

# **PDU FINAL RUN REPORT**

**NREWAMOCO CRADA TASK #4**

**JUNE 14,1996**

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**NREL/Amoco CRADA Task #4**  
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Run number: P960314CF  
Dates of run: 3/14/96 to 4/29/96  
Feedstock: Blended Corn fiber

### 1.0 Executive Summary

The Process Development Unit (PDU) was operated with a corn fiber blend (corn fiber and corn screenings) for approximately six weeks using the Amoco Pretreatment Reactor (APR), the seed fermentation train, fermentation support equipment, three of the main 9000-L fermenters, the fourth fermenter as a kill tank, and distillation and centrifugation systems. The run utilized the recombinant yeast strain, LNHST2, to simultaneously co-ferment glucose and xylose to ethanol. The APR and fermentation equipment operated continuously for 47 days from March 14 through April 29. Near the end of the run, an ethanol recycle stream was added to the first 9000-L fermenter to raise the ethanol concentration to 4.5%.

The APR was operated continuously throughout the six week period with three major shutdowns (one day or longer). Within this period was a 16 day run with only 12 hours of downtime. Throughout most of the run there was a gradual decline in pretreatment performance, shown by decreasing monomeric sugar concentrations in the APR product. Glucose yields (from total soluble glucose) in the pretreated slurry remained relatively constant at 60%—80%, which would be expected from complete starch hydrolysis. Xylose yields (from total soluble xylose) ranged from 100%—70%, declining throughout the run. Carbon balance data on three pretreatment samples were within  $\pm 10\%$  of complete closure, although individual component carbon balances were more variable.

Monomeric glucose was completely utilized during the fermentation, however, little or no xylose was converted to ethanol. This may be caused by a combination of the inhibitors produced during pretreatment and ethanol. Ethanol concentrations reached 4.5% during the early part of the run in the final 9000-L fermenter, but decreased to 3.5% as sugar concentrations dropped in the pretreated feed. Ethanol process yields (based on C6 sugars and xylose) at 40% were low because of unconverted xylose, oligomeric glucose, and cellulose. Metabolic ethanol yields were generally near 75%. Approximately 80% of the products from the sugars were accounted for from mass balance data.

A bacterial contaminant (*lactobacillus*) was detected throughout the run, but only two major outbreaks occurred that required the use of antibiotics. The contaminant consumed arabinose, producing lactic and acetic acid, and did not appear to have a major impact on glucose fermentation. Although, there was already little xylose conversion, these high acid levels (each at 10—20 g/L) would have certainly inhibited any xylose conversion.

Many of the objectives for this run were successfully met, such as, steady state operation of the APR and fermentation train for a period of two weeks, elimination of continuous inoculation, adequate production of sugars by the APR, achieving target solids concentration (25%) and residence times (36 hours per fermenter) in fermentation, and collecting 5.8 tons of dry product for animal feed testing. The ethanol recycle stream was not used to raise ethanol concentrations to 8.0% as originally envisioned because of concerns about ethanol inhibition.

## 2.0 Introduction

The primary purpose of this run was to demonstrate continuous operation of the PDU using the APR for pretreatment and the Purdue recombinant yeast (LNHST2) for simultaneous saccharification and co-fermentation (SSCF) of glucose and xylose. In addition, the run was to prove steady state operation at a high solids level (25%), demonstrate adequacy of the kinetics model, prove yeast growth is sufficient to eliminate inoculum addition, and provide representative product for animal feed testing. The run follows the successful demonstration of LNHST2 performance in batch culture in the PDU fermenters in Task 3. This run showed some conversion of xylose to ethanol at a 25% solids concentrations and laboratory work showed that it should be possible to eliminate continuous inoculation. Before the start of Task 4, an automated system to convey feedstock to the APR was completed. This eliminated manual addition of feedstock from barrels to the APR feed hopper.

## 3.0 Pilot Plant Operations

Operation of the pilot plant began on March 14 and continued until April 29. The new automated feed system, the APR, fermentation, distillation, and centrifugation equipment were used. Operating conditions were decided before the run and are presented in subsequent sections. Additionally, a run history and significant operational notes are presented.

### 3.1 Procedures and Operating Conditions

#### 3.1.1 Feed Handling/Pretreatment Operating Conditions

This run used a blended feedstock of corn fiber and cracked corn in a 8.5 to 1.0 wet weight ratio (3.9 to 1.0 dry weight ratio assuming solids concentrations of 40% and 87% for corn fiber and cracked corn, respectively). Corn fiber and cracked corn were obtained from a Casco corn wet-milling facility (Cardinal plant, Ontario, Canada) and blended, frozen and shipped to the PDU in 55-gal drums in a refrigerated trailer.

Figure 1 shows APR operating conditions. Acid concentration was calculated from flow rates and the acid concentration in the acid feed tank. pH of APR samples were measured with a pH probe after cooling the sample to room temperature. The operators attempted to maintain constant operating conditions, however, there were some variations in the pH from the target 0.9. Some of the variations in pH were due to the measurement procedure. A written standardized was developed midway through the run. Temperature also drifted up; this will be discussed in section 4.2.1.

A new automated feed system was used this run to reduced operator labor. Feedstock was dumped into the PDU feed hopper, which could hold four barrels of material. When the APR feed hopper was low, a low level switch actuated the system. The PDU feed hopper delivered material to the belt conveyor that then dumped into the flexible screw conveyor. The flexible conveyor filled the APR feed hopper until actuation of a high level switch shut down the system.

#### 3.1.2 Fermentation Operating Conditions

Operating conditions for the seed train are shown in Table 1. LNHST2 was grown by successive transfers from a small shake flask to a larger shake flask, and then to the 20-L, 160-L, and 1450-L fermenters, respectively. There was no pH control in the shake flask. pH was controlled at 5 with 3.0 M NaOH in the 20-L and 160-L fermenters and with 50% NaOH in the 1450-L fermenter. A 20% (w/w) inoculum was transferred from the 1450-L fermenter directly into the first 9000-L fermenter. No seed hold tanks were used and no additional inoculum was added to the fermentation during this run.

Fermentation conditions in the 9000-L fermenters are also presented in Table 1. Corn steep liquor (CSL) and enzyme additions were made to only the first 9000-L fermenter. pH was controlled using 50% NaOH. Level was controlled in the 9000-L fermenters (at 3500 L) to maintain a residence time of 36 hours in each vessel. Solids concentrations (as total solids from corn fiber only) were generally 25%—28% after dilution of the pretreated corn fiber by enzyme, CSL, and sterile water (additional water was required to achieve the target solids concentration). Glucoamylase was batched into the first 9000-L fermenter daily at an approximate loading of 2 IU/g starch in the raw feedstock.

### 3.2 Run History

A time line for this run is shown in Figure 2. Operation of the APR began on March 15 and continued until April 29. Significant downtime (one day or longer) is shown in the figure. Problems that caused downtime will be discussed in the operational notes (section 3.3).

Table 1. Fermentation Operating Conditions For Seed Train and 9000-L Fermenters

Operating Condition	Flask #1	Flask #2	20-L	160-L	1450-L	9000-L
Temperature (°C)	30	30	30	30	30	30
Agitation (rpm)	150 <sup>a</sup>	150 <sup>a</sup>	150	100	75	100-125
pH	5.0	5.0	5.0	5.0	5.0	5.0
Gauge Pressure (bar)	-	-	0.33	0.33	0.33	0.33
Airflow (vvm)	-	-	0.5	0.5	0.25	0.03 <sup>b</sup>
Residence Time (h)	6 <sup>c</sup>	6 <sup>c</sup>	6 <sup>c</sup>	12 <sup>c</sup>	12 <sup>c</sup>	36 <sup>d</sup>
Media:						
Glucose (%)	2	2	2	2	2	-
Peptone (%)	2	-	-	-	-	-
Yeast Extract (%)	1	-	-	-	-	-
CSL (%)	-	1	1	1	1	1
Antifoam (corn oil, mL/L)	-	-	0.5	0.5	0.5	-
Enzyme (IU/g cellulose)	-	-	-	-	-	5
Glucoamylase (IU/g starch)	-	-	-	-	-	2

<sup>a</sup> laboratory shaker agitation

<sup>b</sup> air added to maintain a positive pressure in vessels only when needed

<sup>c</sup> typical incubation times

<sup>d</sup> per 9000-L vessel

<sup>e</sup> substrate was pretreated corn fiber blend

The 20-L fermenter was inoculated on March 16 and used to sequentially inoculate the 160-L and 1450-L fermenters. The first 9000-L fermenter was inoculated at 12:30 on March 17. Transfer to other 9000-L fermenters began after level in the first fermenter was above 7000 L, after which the level in the first fermenter was dropped to 3500 L. Operation of the seed train is not shown in Figure 2, since this equipment operated only twice during the run.

The 9000-L fermenter operated in a continuous mode except during loss of pretreated feed. The most significant occurrences were on March 23 (run time 120—200 hours) and March 26 (run time 950—1025 hours). When

APR operation was restored, the fermenters were returned to continuous operation. Although, low level contamination **was** detected throughout the run, it **only** became **a** significant problem on two occasions. The first occasion occurred near April **4** (identified by rapidly raising acetic and lactic acid concentrations and bacterial cell counts), and was controlled by addition of penicillium to all fermenters followed by addition of penicillium to only the **first** 9000-L fermenter. Lactrol (virginiamycin) **was** used after the penicillium supply **was** exhausted. **On** April **15** **an** inadvertent large dose of Lactrol was added to the fermentation, however, after this, bacterial cell counts decreased and contamination was not a further problem. A large increase in bacterial cell counts occurred at the end of the run and was a major contributing factor to ending the run.

Both penicillium and Lactrol attack gram-positive bacteria, such as the lactobacillus contaminant common in these fermentations. But there was not enough data to judge the relative effectiveness of the different antibiotics. Since Lactrol appeared to be effective at reducing and eliminating the contaminant and is easier to obtain, it will be used if necessary during Task 5.

Addition of a recycle stream containing ethanol to the first 9000-L fermenter began on April 20. It **was** intended to raise ethanol concentration in the first vessel to 4.5% **as** a means of controlling contamination. Distilled ethanol (50% w/w) was diluted to approximately 18% in the seed hold tanks. The recycle **stream was** then fed continuously to the first fermenter through the former enzyme addition system. Sterile water addition was reduced **to** maintain the target solids concentration.

### 3.3 Operational Notes

The following is a discussion of significant operational notes and problems that occurred during this run.

#### 3.3.1 Feed **Handling**

This was the first run to use the flex conveyor **and** automated feed system to feed the APR weigh feeder. The system functioned well, although there were some minor problems with feed plugging and resolving Data Acquisition and Control System (DACS) programming problems. The most significant problem was foreign objects getting into the **APR** feed hopper. The most common objects were large wood splinters from the pallets. Several times wood pieces were removed from the entrance to the **APR** after travelling through the feed system. A vibratory screen will be added to remove foreign objects before they can enter the APR feed hopper.

It was difficult during this run to track feedstock **history**. Drums were only labelled with a date or even several dates, that did not provide a clear identification of different feedstock lots. In the future, **drums** will be labelled **with** a lot number and that information will be recorded by APR operators each time they dump **a** load of drums into the feed hopper. Samples from each lot will be sent by Federal Express to both Amoco **and** NREL so that the composition can be determined prior to **using** any given lot.

### 3.3.3 Fermentation

There were no problems with the limited operation of the **seed** train during this run.

Because of the low enzyme flow rate required for this run, a carboy and small peristaltic pump were used to transfer enzyme to the first 9000-L fermenter. Nacin (antibiotic) was added to the enzyme to eliminate any bacteria. The former enzyme tank was used as an additional tank for feeding CSL.

Contaminants were detected in the CSL transfer system and this may be the cause of contamination during this run. The problem may be poor cleaning and sterilization practices on the CSL transfer system. More attention will be paid to maintaining this system during Task 5.

There continued to be a problem with reliable operation of the mass spectrometer (MS) during this run, particularly measurement of ethanol. A vendor representative worked on the system and identified that the ethanol calibration oven was not supplying the correct concentration required for ethanol calibration. The problem was resolved by modifying the oven's heating system to increase the setpoint temperature and thus the ethanol concentration into the range required by the MS. Reliable measurements of exhaust gas composition were taken near the end of the run and used in mass balance calculations.

Coating of the agitator shaft in the first 9000-L fermenter with sodium carbonate during the run continued to be a problem that jeopardizes the agitator's mechanical seal. The problem was previously identified as a reaction of sodium hydroxide used for pH control with carbon dioxide produced by the fermentation. Once during this run, the level in the fermenter was raised to dissolve the buildup. However, this caused chunks of the material to come off and led to plugging problems in the fermenter pumps.

### 3.3.4 Distillation/Kill System

from the column walls falling into the pump inlet. Better cleaning during long term shutdowns will solve this problem.

During this run, the fourth 9000-L fermenter was operated as a kill tank. After distillation, the hot (80°—90°C) bottoms product was fed to the fourth fermenter and held at 80°C for one hour before being discharged to the

sewer. **During** the latter **part** of the run, the bottoms product **was** not cooled after distillation and instead fed into the fermenter while still hot. Both the temperature increase during distillation and subsequent hold of the material for one hour at 80°C in the fermenter were **part** of the approved kill process.

On April 22, a valve on the bottom of the fourth fermenter was accidentally left open during transfer of bottoms product to this tank. About 1500 kg of material **was** discharged to the floor and collected **in** the neutralization tank. Since this bypassed the normal kill procedure conducted in the fourth fermenter, this material was killed by addition of bleach to the neutralization tank before discharge to the sewer. The procedure **has** been revised to more clearly direct the operator to close the valve before filling the fourth fermenter.

### 3.3.4 Centrifugation

The centrifuge was operated to separate solids from the distillation bottoms for testing **as** animal feed. The pilot plant used a Sharples P-3000 continuous decanter, however, this may not be **an** appropriate unit for a commercial plant. Numerous problems were encountered with plugging of lines, flooding of the centrifuge, **and** high torque **alarms** on the centrifuge. These problems required a **significant** amount of operator time **and** intervention to resolve **and** correct. Typical **total** solids concentrations in the cake were **22%**. This **was** lower than hoped, and after consultation with the equipment manufacturer, a larger backdrive motor **was** ordered to increase solids concentration **and** recoveries **and** eliminate the high torque **alarms**.

## 4.0 Key Results

The following sections presents **key** results obtained during operation of the pilot plant.

### 4.1 Feedstock

The composition of corn fiber blends used in this run (P960314CF) as well **as** in earlier runs are shown in **Table 2**. Some of the analysis performed by the outside laboratory are questionable. Note the high glucose content of the first three samples (Mar. 15, 16, and 17) and three samples near the end of the run (Mar. 16, April 22, **and** Lot 3). These results are inconsistent with sample analysis from the middle of the run **and** repeats done by Anioco Analytical **and** the NREL CAT (Chemical Analysis **and** Testing) Team. In particular, the analysis of lot 3 **from** NREL **and** Anioco agree, but are significantly different from the outside laboratory. The questionable results will not be used for **mass** balance and yield calculations. Feedstock composition was likely very close to the middle values throughout the run. Follow up testing during **Task 5** will be used to firmly establish feedstock composition.

### 4.2 Pretreatment

#### 4.2.1 Sugar Concentrations and Yields

Figure 3 shows monomeric and total soluble glucose concentrations in APR samples **as** a function of run time. Total soluble glucose is somewhat stable throughout the run (averaged 332 mg/g TS with a one standard deviation of 29). Monomeric glucose was typically 50%—70% of the total soluble glucose, except **during** periods of extremely poor performance when the ratio **was** in the 20%—40% range.

**Table 2. Corn Fiber Composition**

Lot #	Used In	Source	Moisture (%)	Glucose (%)	Xylose (%)	Gal. (%)	Ara. (%)	Mannose (%)	Lignin (%)	ASL (1) (%)	Ash (%)	Ext. (2) (%)	Starch (3) (%)	Protein (4) (%)	Analysis Source
1	P950310CF	Casco	60	33.4	23.7	3.9	15.5	0.1	6.5	3.4	1		15.7		CAT
	P950425CF	GTC	57.6	41.6	21.2	7.7	12.8	0	7.8	7.8	0.9		24.9	10.4	PDU
2	P950425CF	GTC	54.1	39.9	21.5	7.6	12.2	0	8.5	8.1	0.9		25.6	11.6	PDU
1	P951101CF	Casco	61	44.7	13.3	3	12.5	0.3	4.5	2.4	1	12.4	18.4	9.1	CAT/Out(7)
2	P951101CF	Casco	58	35.5	19.8	4	16.1	0	6.2	5	0.9	9.8	12	10	CAT/Out
3	P951101CF	Casco	57	37.4	19.6	4	15.8	0	6.1	5	0.7	9.1	14.5	9.2	CAT/Out
	Cracked Corn	Casco	13.3	93.1	2.2	0.5	2	0	2	2.2	0.1	6.7	75.7	4.65	CAT/Out
	Blend 1:4.5	Casco	44.5	62.3	13.7	2.4	10.7	0	2.7	4.2	0.9	11.3	41.3		CAT/Out
	Blend 1:8.5	Casco	54	48.3	15.1	3.2	11.1	0	5.5	4.7	0.7	9.1	41.7		CAT/Out
	Calc. 1:8.5 Blend	Casco	55.1	47.6	16.1	3.3	13.2	0	5.3	4.4	0.8		28.3		CAT/Out
15-Mar	P960314CF	Casco	52.6	62.2	11.8	2.3	9.5	0	3.3	3.8	0.9	10.3	19.9	9.2 (5)	CAT/Out
16-Mar	P960314CF	Casco	53.7	62	12.7	2.5	10.1	0	3.3	4	0.9	10.1	13.9	8.4	CAT/Out
17-Mar	P960314CF	Casco	54.3	62.1	12.9	2.6	9.9	0	3.1	4.2	0.8	10.6	18.9	9.1	CAT/Out
28-Mar	P960314CF	Casco	53.6	42	19.3	3.8	12.8	0	3.5	4.7	0.8	8.9	20	7.3	CAT/Out
30-Mar	P960314CF	Casco	53.3	45.9	14.8	3.2	11.8	0	3.5	4.2	0.9	10.1	18.7	7.8	CAT/Out
3-Apr	P960314CF	Casco	52.7	43.1	15.3	3.3	12	0	3.4	4.2	0.8	11.4	19.4	7.5	CAT/Out
26-Mar	P960314CF	Casco	53.8	47	18.9	3.6	11.8	0	2.9	3.6	0.7	12.6	27.8	5.7	CAT/Out
6-Apr	P960314CF	Casco	54.7	42.2	18.2	3.5	12.5	0	3.7	4	0.7	10.6	19.8	7.2	CAT/Out
10-Apr	P960314CF	Casco	55.2	42.8	18.4	3.6	11.9	0	3.9	3.9	0.8	12.3	20.8	7.1	CAT/Out
13-Apr	P960314CF	Casco	54.7	44.4	17.6	3.3	10.4	0	3.5	3.6	0.7	11.1	27.3	6.3	CAT/Out
16-Apr	P960314CF	Casco	54.9	45.8	17.6	3.2	10.9	0	3.7	3.8	0.6	11.9	28.9	6.8	CAT/Out
	P960314CF	Casco		45.3	17.2	2.9	11	0	4	3.7	0.7	11.1	24.9	6.7	CAT/Out
16-Mar	P960314CF	Casco	52.3	66.8	11.5	2.3	8.5	0	2.2	3.1	0.7	9.6		7.6	CAT/Out
22-Apr	P960314CF	Casco	51.9	67.2	8	2.1	7.7	0	2.7	3.1	0.8	8.9		6.7	CAT/Out
3	P960314CF	Casco	54.2	66	10.9	2.2	8.3	0	3.2	3.3	0.7	8.6		7.3	CAT/Out
3	P960314CF	Casco		50.3 (6)	20.2		12.6						37.2		Amoco
3	P960314CF	Casco		45.7	21	4.2	13.5								CAT
22-Apr	P960314CF	Casco		43.7	22.9	4.9	14.7								CAT
22-Apr(8)	P960314CF	Casco		47.5	19.5	4	12.3								CAT

(1) Acid Soluble Lignin

(2) 95% ethanol extraction, extractives include solubilized protein included in the protein number

(3) Starch is also included in the glucose number

(4) Protein calculated from nitrogen content

(5) Beginning with this value, protein is reported on extracted feedstock

(6) Includes some unresolved galactose

(7) CAT/Out - Carbohydrates, ash, and lignin determined by outside laboratory

(8) Same lot but different sample from same day

Figure 4 shows monomeric and total soluble xylose and acetic acid concentrations of APR samples during the run. Like monomeric glucose, there is a downward trend in these concentrations **during** the run. Monomeric xylose was typically 60%—75% of the total soluble xylose, except **during** periods of poor performance.

Yields of **total** soluble glucose and xylose, **and** percent cellulose hydrolyzed during the run are shown in Figure 5. Average feedstock compositions of **46.6%** glucose, **18.4%** xylose, **and 24.5%** starch were used. The calculation of cellulose hydrolyzed assumes complete hydrolysis of the starch. Average glucose, starch, and xylose content of **all** corn blends analyzed during this run were used, excluding the obviously questionable values generated by the outside laboratory. Glucose yields ranged from ~~0%~~— Cellulose hydrolyzed **was** typically between ~~0%~~— Xylose yields were more variable, ranging from ~~0%~~— This data (xylose yields) also shows a gradual decline in pretreatment performance beginning after 200 hours.

#### 4.2.2 APR Correlations

Table 3 presents correlation coefficients for various **APR** parameters. Correlation coefficients (**r**) are used to show **a** linear relationship between variables. The closer the number is to 1.0 or -1.0 the greater the correlation. However, a correlation coefficient of 0.5 indicates that **a** variable accounts for only 25% of the variation in the other variable (determined by squaring **r**). Thus, only values of **r** greater than 0.8 show any significant degree of correlation. The table shows that only acid concentration **and** acid flow rate are highly correlated, with a weaker correlation between acid concentration and pH. These positive correlations would be expected **and** show that changes in acid flow rate are responsible for acid concentration **and** pH changes. None of the other APR variables (not all shown in Table 3) show any significant correlation within the measured range of the variables.

Table 3. Correlation Coefficients for APR Parameters

Variable	Correlated With	Correlation Coefficient
Acid Concentration	Acid Flow Rate	0.92
Acid Concentration	pH	-0.62
	Monomeric Glucose Concentration	0.21
	Monomeric Xylose Concentration	0.33
	Total Soluble Glucose Concentration	-0.08
	Total Soluble Xylose Concentration	0.48
	Acetic Acid Concentration	0.21
pH	Monomeric Glucose Concentration	-0.25
	Monomeric Xylose Concentration	-0.39
	Total Soluble Glucose Concentration	0.13
	Total Soluble Xylose Concentration	-0.48
	Acetic Acid Concentration	-0.29
Temperature	Monomeric Glucose Concentration	-0.26
	Monomeric Xylose Concentration	-0.31
	Total Soluble Glucose Concentration	0.0
	Total Soluble Xylose Concentration	-0.11
	Acetic Acid Concentration	-0.17

Since correlation coefficients only show linear relationship, it is useful to check for non-linear relationships by plotting the data. Figure 6 shows acid concentration plotted against a few other variables. The linear trend is obvious for acid flow rate and pH, but there is no relationships with total soluble xylose or monomeric glucose. Figure 7 shows temperature plotted against some of the other variables and again there are no relationships. This is expected, since as previously mentioned, there is a problem obtaining accurate temperature measurements. If true measurements of process temperature were available, there would probably be some correlations to the product concentrations.

#### 4.2.3 Carbon Balances and Conversions

Material and carbon balances were calculated for three APR samples along with standard deviations for use in uncertainty analysis. Standard deviations (SD) of measurements (random error component only) were calculated from analytical measurements of component concentrations and flow rate measurements. These SD's were measurements of the overall variability of the process (i.e., independent measurements of different samples but at the same controlled conditions) and are not a measurement of repeatability (e.g., a repeat measurement on the same sample). For example, the SD of the feedstock was calculated from three samples taken from the same lot, but over a period of two days. SD of pretreated material composition was calculated from three different samples taken over a 5 min period. SD for flow rate measurements were calculated from DACS data taken over a period of several hours to determine a good estimate of longer term variability. SD for fermentation broth composition was calculated from an analysis of the three independently taken samples from the same vessel.

Errors were combined using the propagation of error formula, which is based on a Taylor Series approximation (methodology defined in standard ANSI/ASME PTC 19.1-1985). Given a function  $F = f(x_i)$   $i=1,2,\dots,n$ , then the SD ( $\alpha_F$ ) of F is given by:

$$\alpha_F^2 = \sum (\partial F / \partial x_i)^2 \alpha_i^2,$$

where  $\alpha_i$  is the SD of  $x_i$ . The 95% confidence interval ( $U_{RSS}$ ) is given by:

$$U_{RSS} = \sqrt{B^2 + (t\alpha_x)^2},$$

where B is the estimate of bias error,  $\alpha_x$  is the random error and t is the t value (from Student t distribution) associated with the degrees of freedom used to calculate  $\alpha_x$ . The t value will be assumed to be 2. The random error  $\alpha_x$  consists of two parts, the process error (associated with the ability to control at a setpoint or repeat the same conditions for a sample as discussed above) and the measurement error. Repeat measurement in the past have shown that measurement error is significantly smaller than process error and so will be ignored. Likewise, bias error will be assumed to be small compared to process error and will be ignored, which reduces the 95% confidence estimate to:

$$U_{RSS} = 2\alpha_F.$$

Table 4 shows percent carbon closure, percent conversion of solid fractions to component sugars, and percent monomeric to total soluble sugar ratio for three APR samples. Samples APR-214 and APR-329 used a feedstock composition that was an average of all blends analyzed during this run, except for the questionable analysis performed by an outside laboratory. This information was also used to calculate standard deviations for blended feedstocks. Sample APR-295 used a feedstock composition of a feedstock sample taken at the same time as the

pretreated sample.

Table 4. Pretreatment Carbon Balance Closure with One Standard Deviation (in parentheses), Conversion of

Component	APR- 214 3/16/96 13:00 (-23.5 hours)	APR-295 4/16/96 13:00 (72I hours)	APR-329 4/29/96 13:00 (1032.5 hours)
<b>Carbon Closure (%)</b>			
Glucose	<b>95.4 (13.6)</b>	96.0 (13.3)	<b>97.0 (13.7)</b>
Galactose	<b>81.8 (15.1)</b>	<b>46.2 (10.0)</b>	<b>88.2 (16.3)</b>
Xylose	85.8 (18.6)	<b>80.5 (19.0)</b>	<b>84.9 (19.1)</b>
Arabinose	<b>83.9 (13.6)</b>	<b>84.5 (14.8)</b>	<b>90.0 (14.6)</b>
Lignin	111.6 (21.0)	<b>113.6 (24.6)</b>	<b>127.1 (25.6)</b>
Overall	94.7 (8.5)	<b>93.0 (8.5)</b>	98.8 (8.8)
<b>Conversion to Total Soluble Sugars (%)</b>			
Glucose	65.3	<b>71.3</b>	<b>67.4</b>
Galactose	<b>79.4</b>	<b>43.7</b>	<b>87.7</b>
Xylose	81.5	<b>76.6</b>	<b>83.5</b>
Arabinose	81.1	80.5	<b>88.7</b>
<b>Monomeric to Total Soluble <del>sugar</del> Ratio (%)</b>			
Glucose	62.8	61.8	<b>72.8</b>
Xylose	<b>65.5</b>	<b>66.9</b>	81.6

Carbon balance closure for all samples are good [except for galactose in **APR-295**] and well w i t h 100% at a 95% confidence interval (two times the standard deviation). Conversions (or total soluble **sugar** yield) is as expected **as** previously discussed. **APR-329** was taken near the end of the run and appears to have been pretreated at **a** greater severity (i.e., greater xylose conversion and monomeric to total soluble **sugar** ratios), which **was** evident from the higher monomeric **sugars** levels recorded near the end of the **run**.

All pretreatment data **and** detailed material balance **printouts** are shown in Appendix A.

4.3 Fermentation

4.3.1 SSCF

The SSCF **train** began operation on March 17 with inoculation of the first **9000-L** fermenter at 10:00 (runtime 0 hours). The third fermenter was filled by **mid-day** on March **19**. The residence time for each fermenter **was** **35—37 hours** and the solids concentration **was** 25%—28%. The **train** operated in a continuous mode **except**

when feed was lost from the APR, at which time operation reverted to batch. As previously identified for the APR, the longest period of continuous operation occurred from March 27 through April 12 (runtime 225—610 hours).

#### 4.3.1.1 Sugar and Product Concentrations

Figure 8 shows monomeric sugar concentrations and LNHST2 cell counts in all three 9000-L fermenters over the course of the run. Cell counts start high because of the large amount of cells in the inoculum and the abundance of glucose available when the fermenter is first inoculated. The cell counts typically drop off when during continuous operation. It is not clear why cell counts appear to increase later in the run. LNHST2 cell counts did not appear to suffer during the period of contamination (runtime 400—700 hours). Although cell counts tend to be variable, it is clear that yeast populations remained in the fermenters and were not washed out. This proves that at a 36 hour residence time and with pretreated material at the severity being produced during this run, yeast growth was adequate to maintain a viable population and consume all the glucose.

Total soluble glucose levels typically remained between 15—20 g/L in the third fermenter (data not shown). The high total soluble glucose level was first noted during Task 3. Several possible explanations are that these sugars are "limit dextrins", which are known in the corn industry as starch oligomers that cannot be converted to glucose by enzymes. They may also be oligomers derived from cellulose being left behind because of insufficient cellulase activity (e.g., low loading, adsorption loss, inhibition).

Glucose is all converted in the first fermenter, while it is difficult from these plots to see any consumption of xylose (xylose results will be presented later). The plots clearly show the consumption of arabinose (particularly in the second and third fermenters) corresponding to heavy bacterial contamination beginning at 400 hours, mid-way during the long continuous operational period discussed above. The bacteria, previously identified as a *lactobacillus*, will preferentially consume arabinose and produce lactic and acetic acid.

Figure 9 shows product concentrations in all three of the 9000-L fermenters. This plot shows the obvious increase in lactic and acetic acid concentrations during the period of heavy contamination, xylitol concentrations increased as well approximately 3—4 g/L. However, the component measured as xylitol is likely arabitol produced by the consumption of arabinose (both components have nearly the same retention time on the HPLC). Ethanol production appears to be unaffected by the contamination. The slow drop in ethanol concentration is probably due to the decreasing sugar concentrations in the pretreated feed during this period (see Figure 3).

The peak concentrations of lactic and acetic acid at about 700 hours (March 15) cannot all be accounted for by arabinose (only about 2/3). There is a drop in xylose concentration at about 700 hours that was likely used for acid production. Previous typing of the contaminant has shown it prefers C5 sugars, in particular arabinose. There was no feedstock change during this period and so xylose content of the feed was not responsible for the drop in xylose concentration. This is confirmed by Table 2 which shows a constant xylose content in the feed bracketing this period.

The concentrations of xylose and ethanol in all three fermenters are compared in Figure 10. The plot clearly shows very little xylose conversion at any time during the run. This is confirmed by nearly the same ethanol concentration in all three fermenters over the course of the run. The only exception seems to be at the beginning of the run where there is an 8 g/L difference between the first and the third fermenters. When the fermenters were put in batch operation (100—200 hours), there was also consumption of xylose in the first fermenter during this period. The lack of xylose conversion is likely due to some type of inhibition. Known inhibitors present are acetic acid, furfural, HMF, and ethanol. However, none of these have been inhibitory by themselves at the concentrations present in the fermentation according to SWAN information provided by Bob Lumpkin. Unknown inhibitors may be created during pretreatment as well as Maillard reaction products from the high levels of glucose and proteins present in this feedstock. The combined effect of all the inhibitors may also be a problem for xylose fermentation.

#### 4.3.1.2 Carbon Balances and Yields

Table 5 shows fractional conversion of the raw feedstock after the combined process of pretreatment and SSCF and SSCF yields for two samples during the run. The average feedstock composition as discussed above was used. The April 3 sample (run time 405 hours) was taken just before high levels of lactic and acetic acid began to appear. The other sample was taken near the end of the run on April 26 (runtime 957 hours) and carbon balances were calculated using both ethanol stoichiometry and measurements of exhaust gas composition.

**Table 5. Conversion of Raw Feedstock and SSCF Yields With One Standard Deviation (in parentheses)**

	4/3/96 10:00 <sup>1</sup> (405.5 hours)	4/26/96 10:00 <sup>1</sup> (957.5 hours)	4/26/96 10:00 <sup>2</sup> (957.5 hours)
Conversions (% consumed/total in):			
Glucose	68.1 (1.5)	71.5 (1.5)	71.5 (1.5)
Galactose	17.4 (1.3)	32.9 (1.5)	32.9 (1.5)
Xylose	12.0 (6.9)	28.2 (8.0)	28.2 (8.0)
Arabinose	26.7 (3.7)	35.8 (4.7)	35.8 (4.7)
Lignin	-35.6 (15.3)	8.9 (12.7)	8.9 (12.7)
Yields (g/100 g C6+C5 consumed):			
Ethanol	35.5 (6.2)	33.5 (6.9)	34.9 (5.4)
Carbon Dioxide	33.9 (5.9)	32.0 (5.4)	24.5 (4.1)
Cell Mass <sup>3</sup>	4.9 (1.3)	4.2 (1.1)	4.2 (1.1)
Glycerol	5.0 (0.9)	2.5 (1.4)	2.5 (1.4)
Xylitol	0.0 (0.0)	1.5 (0.3)	1.5 (0.3)
Acetic Acid	2.7 (0.6)	1.4 (0.3)	1.4 (0.3)
Lactic Acid	2.6 (0.5)	2.2 (0.4)	2.2 (0.4)
Succinic	0.8 (0.2)	0.0 (0.0)	0.0 (0.0)
Total	85.3 (8.7)	77.2 (9.0)	71.2 (7.0)
Ethanol Process Yield (% C6 + xylose)	36.8	41.1	42.9
Ethanol Metabolic Yield (% C6 + xylose)	76.3	73.0	76.2
Overall Carbon Recovery (%)	98.8 (6.7)	88.5 (6.3)	87.0 (6.2)

<sup>1</sup> Carbon dioxide based on stoichiometry

<sup>2</sup> Carbon dioxide based on exhaust gas flow rate and composition

<sup>3</sup> Cell mass concentration assumed to be 5.0 g/L in final fermenter

Conversion of glucose in both samples shows probably complete conversion of the starch along with conversion of approximately 33% of the cellulose. A minor amount of galactose was consumed, but galactose is a very minor component of the feedstock (3.6%). Minor amounts of xylose and arabinose were consumed, particularly for the second sample. Arabinose consumption can be attributed to a contaminant. For the second sample, the ethanol production was more than can be attributed to just glucose, therefore, some xylose was converted to ethanol. This is not true for the first sample and given the large errors, it cannot be said with confidence that there was any conversion of xylose to ethanol. Because of the large variability in measuring xylose in the feed,

there is a large error in the conversion calculation for xylose. The lignin closure is poor for the first sample, but lignin closure for the second sample is adequate and well within a 95% confidence interval that brackets 0%.

The product yields are also shown in Table 5 and are based on product produced from all five- and six-carbon sugars. Cell concentration in the fermenters was assumed to be 5 g/L. This is higher than the value calculated from cell mass measurements, which is suspected to be low (approximately 1–2 g/L). But, seems reasonable when compared to cell mass production on pure sugars at the bench scale, which is too high (approximately 10–15 g/L). As usual it is difficult to account for all of the fermentation products. The best value is for the first sample and would bracket 100 at a 95% confident interval. The second sample is disappointing.

Carbon dioxide measurements of exhaust gas yielded significantly less carbon dioxide than would be predicted from stoichiometry. This may be a problem with the gas flow measurement or composition measurement from the mass spectrometer. The flow measurement was extremely difficult because the fermenter back pressure control valves did not maintain a steady flow. Instead, the flow pulsed at a frequency ranging from 10–20 min and data was collected by the DACS on a 20 min interval. For Task 5, data will be collected more frequently during the period when a carbon balance will be performed on fermentation. Mass spectrometer ethanol measurements showed that approximately 2% of the total ethanol production escapes in the exhaust gas, which seems reasonable compared to bench scale experiments that show no ethanol in the exhaust gas after a cooling water condenser.

As with Task 3, ethanol process yields (theoretical ethanol from total available six-carbon sugars and xylose) were low. This is from little or no conversion of the xylose, the 15–20 g/L of oligomeric glucose (approximately 20% of the total soluble glucose) that is not converted during the fermentation, and cellulose not converted. Some cellulose not converted was expected, however, the lack of soluble sugar conversion represents a significant loss of potential ethanol.

The overall carbon recoveries were less than 100% as expected, but 95% confidence intervals would bracket 100% except for the sample using mass spectrometer data. All fermentation data and detailed carbon balance printouts are shown in the Appendix B.

#### 4.3.1.3 Viscosity Measurements on Fermentation Broth

During this run, viscosity measurements were made on fermentation broth samples taken from each of the three 9000-L fermenters on April 29, the last day of the run. The measurements were made with a Stormer viscometer after generating a calibration curve using standards at 60-, 200-, and 400-cP. The results were 259-, 179-, and 149-cP for samples from the first, second, and third fermenters, respectively. This confirms visual observations of broth thinning down the fermentation train.

#### 4.3.1.4 Contamination

A lactobacillus contaminant was detected in the first and second 9000-L fermenters at 168 hours, however, no significant production of by-products were seen until 400 hours. The source of the contamination has not been positively identified, but samples taken from both corn steep liquor (CSL) tanks three days after contamination was first detected in the fermenters contained lactobacillus and yeast. Changes were made to the tank turnaround procedure to include a thorough wash step and more frequent flush and sterilization of the transfer lines. After these changes were made, no contaminants were detected in these tanks. The transfer line from the CSL tanks to the first fermenter will be modified for Task 5 to improve sterilization of the line.

As mentioned above, lactic and acetic acid began to increase in the fermenters around 400 hours. Prior to that, at 130 hours into the fermentation, the APR went down for three days. Without glucose, the yeast stopped producing ethanol and carbon dioxide. As the general health of the yeast decreased because of no available glucose and slow utilization of the xylose, the environment improved for the bacteria. The contamination was

eventually brought under control with a large dose (200 mg/L) of the antibiotic Lactrol (virginiamycin) and operated relatively contamination free (i.e., low bacterial cell counts) until near the end of the run.

An ethanol recycle stream was added to the first fermenter on April 20 (runtime 825 hours) to maintain an ethanol concentration of 4.5% in an attempt to control contamination. Even at the higher ethanol level, severe contamination reappeared at the end of the run (runtime 975 hours). The increase in bacteria coincided with a decrease in APR performance that was associated with blowbacks (at 970 hours) and the APR being taken down to replace the screws. Pretreated samples at this point contained large chunks of inadequately pretreated material. Contamination checks of this material did not show any bacteria, but a filamentous fungus was isolated. Generally, filamentous fungi are more sensitive to heat, so it was surprising to see a fungus rather than bacteria in the sample. This does not positively identify inadequately pretreated feedstock as the source of contaminants in the fermenters, but the possibility exists given the drop in the APR performance. However, contaminant concentrations were increasing before the APR blowback that resulted in the unit being taken down.

### 4.3 Supporting Bench Scale Work

The following sections report on bench scale work carried out before and during Task 4.

#### 4.3.1 Continuous Inoculum Study

Before Task 4, a shake flask study was conducted to determine if yeast growth rate was sufficient to eliminate continuous inoculation. The results showed that at a 36 h residence time with a pretreated corn fiber blend, yeast growth was sufficient to achieve a steady state and consume all the available glucose. Based on these results, cell counts and sugar concentrations were closely monitored during Task 4 startup and the results verified that the fermentation did not need continuous inoculation. A study of cell count versus viable cell counts verified that viable cell counts underestimate actual cell counts and cell mass. The results of this bench scale study are reported in Appendix C.

#### 4.3.2 Strain Evaluation During the PDU Run

One concern with continuous fermentation is the loss of desired properties possible resulting from the extended number of times the organism reproduces. This is important when mutagenized or recombinant organisms are used because of the potential to revert back to the original state of the organism. The advantage with LNHST2 is that the genetic information has been integrated into the chromosome, making the organism more stable and less subject to reversion.

During Task 4, the yeast was consuming little or no xylose, therefore, the strain was checked for the possible loss of the xylose fermenting genes. Other yeast cells were also present in the fermenter that were morphologically very different from the original LNHST2. This unknown yeast could be a contaminant or could be a revertant of LNHST2. The unknown yeast and LNHST2 were isolated from the first 9000-L fermenter after 1000 hours of operation. The two strains were grown in yeast extract, peptone and glucose (YPD) medium. Their growth rates were compared to a fresh culture of LNHST2 taken from our culture stock. The growth curves and maximum growth rates ( $\mu$ ) are shown in Figure 11. The two LNHST2 cultures exhibited the same growth rates, but the unknown yeast grew much quicker. The higher growth rate might be expected from a wild yeast contaminant (not metabolically burdened with the xylose-fermenting genes) and could pose a threat to the fermentation because of its higher growth rate.

Each of the three different yeasts were transferred to YPD<sub>X</sub> medium (yeast extract, peptone, 60 g/L glucose and 30 g/L xylose) and a media with 1.0% CSL and 60 g/L and 30 g/L glucose and xylose, respectively, for further evaluation. Figure 12 shows the concentrations of xylose and fermentation products when ethanol had reached its highest concentration (glucose was fully utilized by all strains within 24 hours). Both LNHST2 cultures exhibited the same fermentation performance. This shows that the LNHST2 strains were stable after 1000 hours

of operation and that loss of the xylose fermenting genes were not responsible for poor xylose utilization during Task 4. The unknown yeast did not ferment xylose, but did produce ethanol at 90% of theoretical. Because the morphology is so different from LNHST2, the yeast is likely a contaminant. The strains have been sent to Nancy Ho (the developer of LNHST2) at Purdue University for further evaluation. Preliminary results showed that LNHST2 isolated from the fermenters after 1000 hours of operation were still able to ferment xylose as effectively as the original culture. Nancy Ho also believes the unknown yeast is a contaminate, but this has not been proven.

#### 4.3.3 LNHST2 Screening Study

A two-level, three factor, half-fractional factorial experiment was designed as a screening study to examine the effect of nutrient sources (CSL - 1% w/v versus 2% w/v, and 2% w/v CSL versus YEP), initial total sugar concentration (82.6 g/L versus 115.5 g/L combined monomeric glucose and xylose), and acetic acid concentration (3.3 g/L versus 6.2 g/L) on ethanol metabolic and process yields, cell growth, and sugar consumption. A second two-level, four factor, half-fractional factorial experiment was designed and carried out to examine the effect of CSL (1% w/v versus 2% w/v), initial total sugar levels (74.4 g/L versus 105.8 g/L combined glucose and xylose), pH (5 versus 6.5) and caustic (ammonium hydroxide,  $\text{NH}_4\text{OH}$  versus sodium hydroxide,  $\text{NaOH}$ ) on organism performance. A detailed report of this work is given in Appendix D.

Both studies were carried out in shake flasks using hydrolyzate separated from a mixture of pretreated corn fiber and corn screenings. The pretreated material was taken on April 21 (runtime 840 hours) and appears to be well pretreated (xylose yield is approximately 85%). The amount of hydrolyzate used was equivalent to the amount present in a 25% total solids fermentation. The pH of each flask was monitored and adjusted to pH 5 with 3 M  $\text{NaOH}$  in the first experiment and to pH 5 or 6.5 with either  $\text{NaOH}$  or  $\text{NH}_4\text{OH}$  in the second experiment. The flasks were inoculated using the standard two-stage inoculation procedure. Temperature was controlled at 30°C and the agitation was set at 150 rpm.

The first experiment showed a significant negative effect of acetic acid on xylose conversion and ethanol process yield. There was no improvement in fermentation performance by increasing CSL from 1% w/v to 2% w/v. Adding YEP as the nutrient source also did not increase xylose conversion or ethanol process yield.

The second experiment examined higher pH and if using  $\text{NH}_4\text{OH}$  to neutralize and control the pH would improve the ethanol process yield and xylose conversion previously observed. Xylose conversion was improved with both  $\text{NaOH}$  and  $\text{NH}_4\text{OH}$  at a pH of 6.5. However, greater xylose conversion did not translate into a greater ethanol yield when  $\text{NH}_4\text{OH}$  was used due to the production of higher levels of the by-products (xylitol [may include arabitol], glycerol, acetic acid, and cell mass). The process yield was better in all cases when  $\text{NaOH}$  was used for pH control, and was slightly better at pH 6.5 than pH 5. Again, at the higher pH, more by-products were produced than at the lower pH.

An interesting phenomena observed in this experiment was the utilization of arabinose at the higher pH. This could have been due to the presence of a contaminant, however, contamination was not observed in any of the flasks. The utilization of arabinose by yeast could potentially minimize contamination problems observed in the PDU by eliminating the primary sugar used by the bacterial contaminant.

#### 4.4 Centrifugation

Approximately 25 tons of material at 22%—24% solids concentration (5.8 dry tons) was collected for animal feed testing. Part of this material may not be acceptable, because it was collected during the early part of the run in which the material was killed at 125°C instead of 80°C. The higher temperature produced a darker product which may contain additional degradation products that may not be suitable for animal feed. Approximately 80% of the material is acceptable. Typical recovery factors in the cake were 50% of the total solids (dissolved and insoluble) and 80% of the insoluble solids. A new backdrive will be installed during Task 5 in an effort to

improve solids concentration in the cake.

#### 4.5 Kinetic Modelling

Steady state operation of the SSCF train was compared with performance predicted by the kinetic model. The kinetic model was run twice with feed compositions and flow rates from Task 4, although it was necessary to use hydrolyzate and SSCF broth compositions from different times during the run. Component concentrations in hydrolyzate were from the April 16 (13:00) APR carbon balance data. Enzyme, CSL, and dilution water flowrates were assumed to be at their respective set points. Component concentrations in the first and second fermenters were from April 3 (2:00) data. Component concentrations in the third fermenter and glucose concentration in the enzyme were from the April 3 (10:00) SSCF carbon balance data. In the first kinetic run, parameters developed to match the Task 3 batch fermentation were used. In the second kinetic run, the ethanol inhibition constants were changed from ones developed with Task 3 data ( $K_{E,G}=29.286$  g/L and  $K_{E,Z}=29.286$  g/L) to ones developed using data from the shake flask experiments done at Purdue ( $K_{E,G}=73.7$  g/L and  $K_{E,Z}=21$  g/L).

Figures 13, 14, 15, and 16 show predicted and measured ethanol, xylose, glucose, and cellulose concentrations in the SSCF train. Figure 13 shows the ethanol concentrations in each of the three fermenters. In all cases, the model predicts higher ethanol concentrations than measured values. The difference was less when using the Purdue ethanol inhibition constants (determined from shake flask experiments with different level of ethanol and acetic acid). Figure 14 shows assumed xylose concentration in the mixed stream entering the first fermenter and predicted and measured value in the each of the other three fermenters. The measured concentration in the first fermenter is higher than the concentration entering it, because the compositions were measured at different times. Xylose utilization in all three fermenters is overpredicted, so inhibition by components other than glucose and ethanol should be further investigated. The overprediction of xylose utilization caused the predicted ethanol concentrations to be higher than the measured values. Figure 15 shows glucose concentration in the three fermenters. Glucose concentration in the feed was 62 g/L. The model essentially shows complete utilization of the glucose. However, glucose measured by our normal HPLC method (Biorad columns for first and second fermenter only) gives numbers that are biased high due to interference from oligomeric sugars. Corresponding YSI numbers for the fermenter are also shown. The glucose concentration in the third fermenter was measured by the PAD system, which does not suffer from the interference and therefore more closely matches the YSI number. Glucose measured by YSI shows more complete utilization of the glucose and thus more closely matches the predictions. Figure 16 shows cellulose hydrolysis in the SSCF train. Cellulose concentrations are only measured in samples used for mass balances, so the measured concentrations were not available for the first and second fermenter. The data shows more cellulose hydrolysis than predicted, but probably well within the range of measurement accuracy.

The kinetic model predicted ethanol concentration in the third fermenter within 20% of the measured value. However, more work is needed to characterize xylose utilization and cellulose hydrolysis to improve the accuracy of the predictions. Task 5 will focus on obtaining complete mass balance data at the same time to better characterize the entire process.

#### 5.0 Review of Run Specifications

The following is the list of criteria for success defined in the Task 4 run specification, and a short discussion of how each of these criteria were met.

1. Operate the APR and SSCF train at steady state for a period of 14 days with no interruption longer than 6 hours and the total of all interruptions should be no longer than 12 hours out of the best 14 consecutive days. The SSCF train must be contamination free during this period.

The APR and fermentation train operated **from** March 27 to April 12 (16 days) with only 12 hours of downtime with **6 hours** as the longest period of downtime. **The** sugar concentrations in the pretreated feedstock showed a small gradual decline over **this** period and this was mirrored by **a gradual** decline in ethanol concentrations during **SSCF**. **A** contaminant was present **during** the last few days and consumed arabinose, but this did not have **a** noticeable effect on ethanol concentrations or products normally produced by the yeast. **This** could be considered contamination free according to the run specifications, although probably more than 10% of the living cells were contaminants.

3. **Run** three stages of **SSCF** at a total solids concentration of at least 25%.

Three stages of **SSCF** were **run** at a solids concentration ranging from 25%—28%.

4. Maintain at least 8 weight % ethanol in the **feed** to distillation.

Because of concern about ethanol inhibition of xylose metabolism at such high ethanol concentrations as shown by the Purdue experiments, this run specification was eliminated.

5. Prove that yeast growth is sufficient in the system so that additional inoculum is not required.

**SSCF** operated for six weeks without the need for additional inoculum. Cell growth was sufficient to maintain **a** steady population on pretreated feed produced by the APR **during** this run and at **a** 36 hour residence time.

6. Close overall mass and carbon balance around pretreatment **and** the **PDU** to within **15%**. Close component balances to **within** 25%. Use measurements of process offgas composition to close material balances.

Overall **carbon** balances were closed **to** within **15%** and most component balances were closed to within 25% (**one** exception was galactose on **an** APR sample). Process offgas measurements were used for both **APR** flash vapor **and** fermentation exhaust **gas**.

7. Compare the **performance** of the kinetics model with Task 4 data and see if the ethanol production rate is Within **20%** of the predicted value.

**Actual** ethanol production was within 20% of the predicted value when ethanol inhibition constants generated **from** Purdue data were used. But, the predicted xylose consumption and ethanol production were greater than **actual** values.

8. Collect fermentation solids and wash so that sulfate levels in the wash water are no higher than 0.1 **wt. %**. Save solids for **future** testing as **animal** feed.

Approximately **5.8 dry** tons of solids were collected **for animal** feed testing. There was no washing of the solids and sulfate levels in the cake were approximately **0.7 wt. %**.

## 6.0 Major Problems and Post Run Issues

The following is a list of significant problems and issues encountered during this run and steps that will be taken to resolve these problems before the next run.

- Flex conveyor and automated feed system problems  
DACs programming and operation problems were resolved during the run. Between Task 4 and 5, a vibratory screen will be added between the flex conveyor and the APR feed hopper to remove foreign objects in the feed.
- Identification of feedstock lots  
The supplier is now identifying lot numbers and labelling each drum with the appropriate lot number. APR operators will record lot numbers of each drums dumped into the feed hopper.
- Questionable feedstock composition measurements  
An outside laboratory generated several questionable values for feedstock composition. These values are being followed up with the laboratory as well as some additional parallel testing by NREL's CAT Team and the outside laboratory. If the issue can not be resolved, future analysis for Task 5 will be performed by NREL.
- Exhaust gas flow measurements for carbon balance calculations  
Because of the pulsed exhaust gas flow produced by the fermenter back-pressure control values, it was difficult to get an accurate measurement of exhaust gas flow. For Task 5, data will be collected at a greater frequency (1 min instead of 20 min) to allow a more accurate calculation of the average flow rate.
- Contamination of CSL system  
More rigorous cleaning and sterilizing of the CSL transfer system will be implemented during Task 5 to avoid contaminating the fermentation. The transfer line will be modified to improve sterilization.
- Poor centrifuge performance  
The centrifuge separation efficiency and cake solids concentrations were lower than expected. After consulting with the equipment manufacturer, a new back drive motor was ordered and will be installed for Task 5.
- Lack of xylose conversion  
To date, little conversion of xylose to ethanol has been achieved in the PDU. This may be due to inhibition by a variety of substances, including ethanol and pretreatment products. This issue should more appropriately be investigated at the bench scale. A continuous bench-scale system will be set up and run parallel with Task 5 to investigate this issue. However, lack of xylose conversion is not expected to significantly affect the quality of the feed generated for animal testing.
- High levels of oligomeric glucose remaining at the end of the fermentation  
As previously identified during Task 4, there are high levels of oligomeric glucose (15—20 g/l, approximately 20% of the total soluble glucose) remaining at the end of the fermentation. During Task 5, work will be done to identify the source of these sugars.
- Lack of mass balance integration for all unit operations  
Data collected up to this point well characterizes individual unit operations (i.e., pretreatment and fermentation). During Task 5, samples will be collected to allow better characterization of the entire process.

- Fermentation cell counts

The use of cell counts to calculate cell mass is still giving amounts that are lower than expected. During Task 5, a correlation will be developed using LNHST2 for estimating cell mass from hemacytometer cell counts.

## 7.0 Summary

LNHST2 was initially grown in the seed fermentation train using the 20-L, 160-L, and 1450-L fermenters. The APR and fermentation equipment were successfully operated for a period of six weeks. Although the APR had several major downtimes (one day or longer), there was a continuous 16 day stretch of nearly continuous operation. A steady state was achieved during this time in which ethanol concentration in the fermenters tracked average sugar production from the APR.

Although contaminants were detected in the main fermenters, antibiotics were used to successfully control major outbreaks.

Spent fermentation broth was distilled as part of the kill step and to recover ethanol for use in a recycle stream. The column bottoms were sent to centrifugation to recover the solid product and approximately 5.8 tons of dry product was collected for animal feed testing.

Most of the specifications for this run were successfully achieved, including operation at steady state for 14 days, adequate conversion of feedstock to sugars during pretreatment, sustained operation above 25% solids in SSCF at a total residence time of 36 hours in each fermenter, adequate closure of mass and carbon balances, elimination of continuous inoculation, and collection of product for animal feed testing. Use of a recycle stream to achieve 8.0% ethanol to distillation was not done because of concerns about ethanol inhibition. However, a recycle stream was used to increase ethanol concentration in SSCF to 4.5%. The most serious concerns are little or no xylose conversion to ethanol in the PDU and high levels of unconverted oligomeric glucose. Both of these issues need to be investigated at the bench scale.

## 8.0 Acknowledgments

The following staff members contributed either full or part-time help to the operation of the plant during this run: Brian Boynton, Nancy Combs, Kevin Enomoto (electrical problems) Rick Houston (SWAN), Kelly Ibsen, Ed Jennings, Tim Johnston, John Lesko (SWAN), Bob Lyons, Sam McWilliams (SWAN), Tim Plummer, Dana Rice, Cindy Riley, Mark Ruth, Dan Schell, Larry Schwartz (SWAN) and Ian Thompson (DACS problems). Analytical support was provided by Larry Brown, Tina Ehrman, Fannie Posey Eddy, Jim Hora, Netta Ingle, Janet Pride, Ray Ruiz, and David Templeton. Christos Hatzis supplied the original material balance spreadsheets that were subsequently modified for use with PDU data. This report was put together with written contributions from Nancy Combs, Mark Ruth, Dan Schell, and Susan Toon.

Figure 2. PDU Task 4 Run History

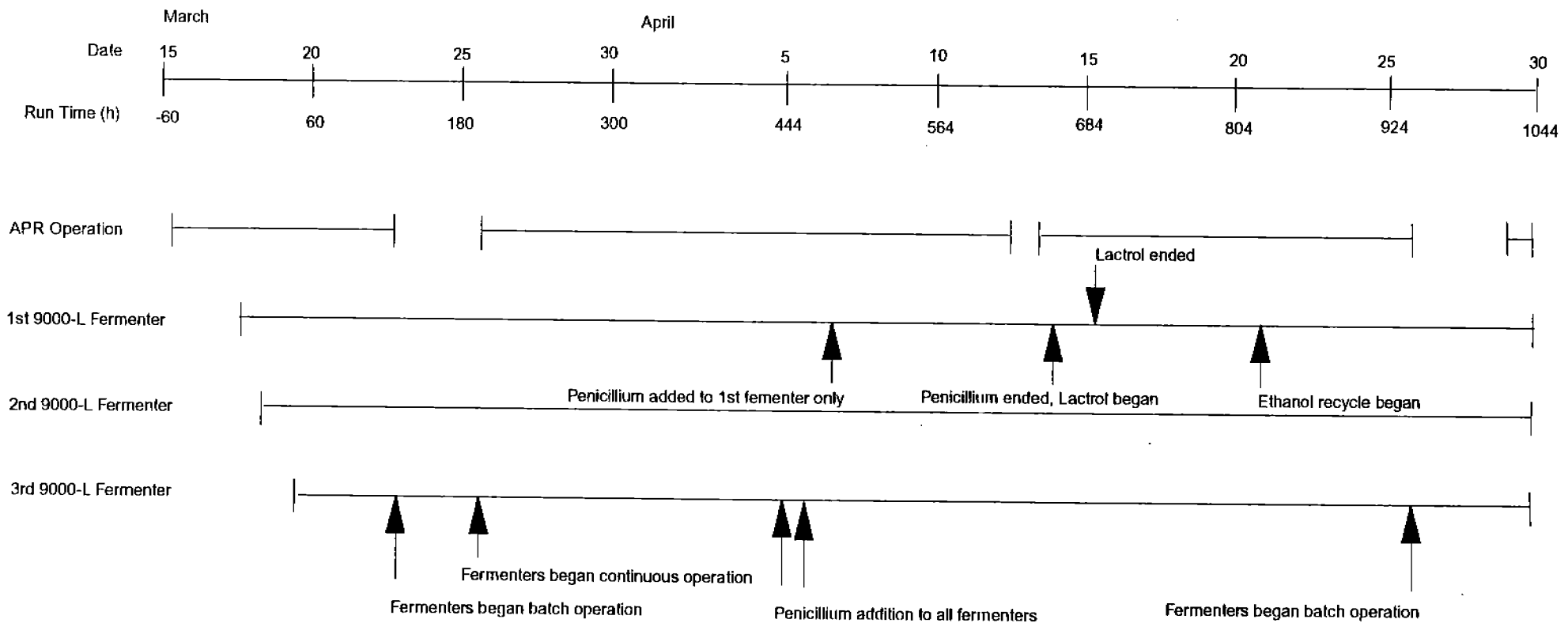


Figure 3. Glucose Concentrations in APR Samples

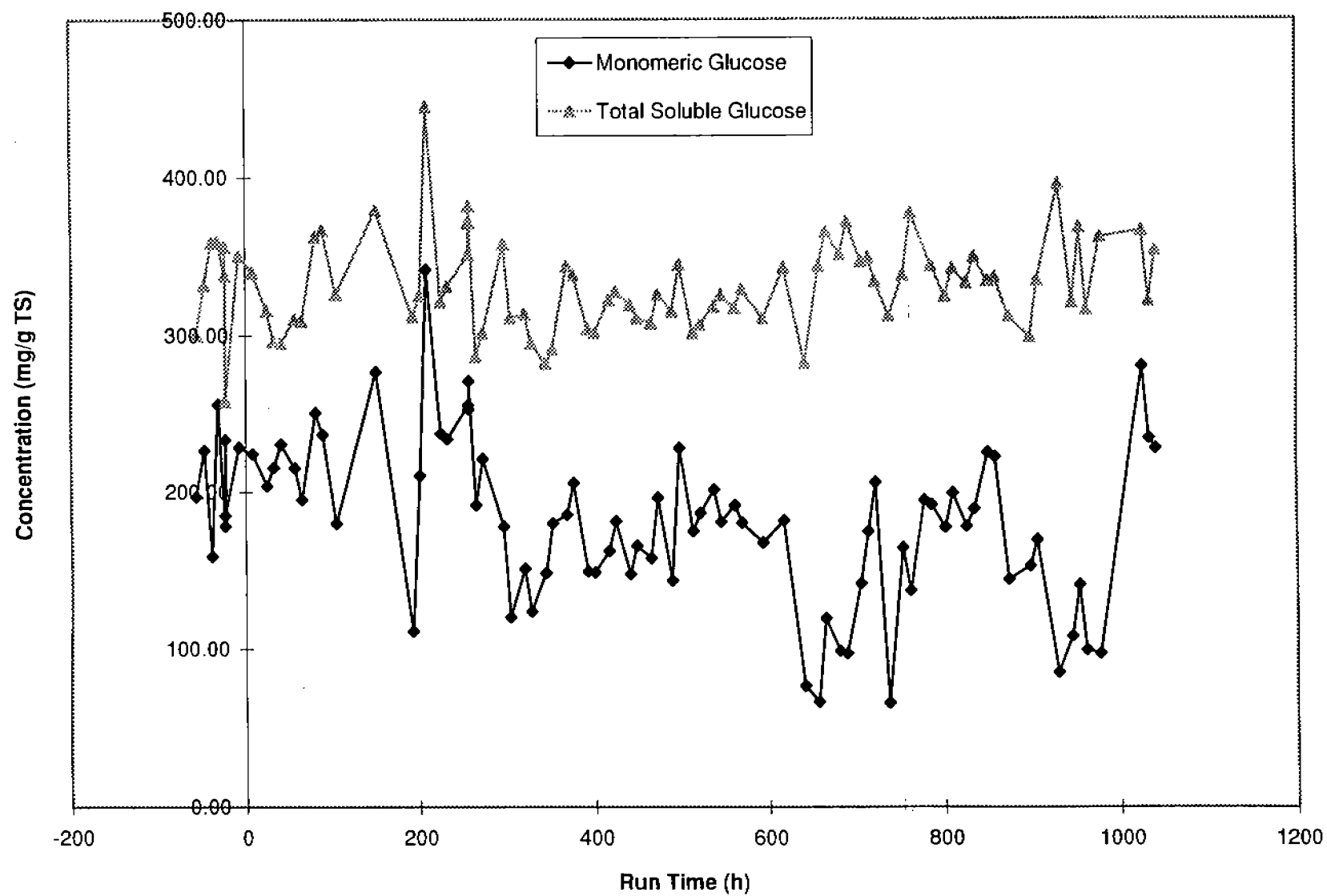


Figure 4. Xylose and Acetic Acid Concentrations in APR Samples

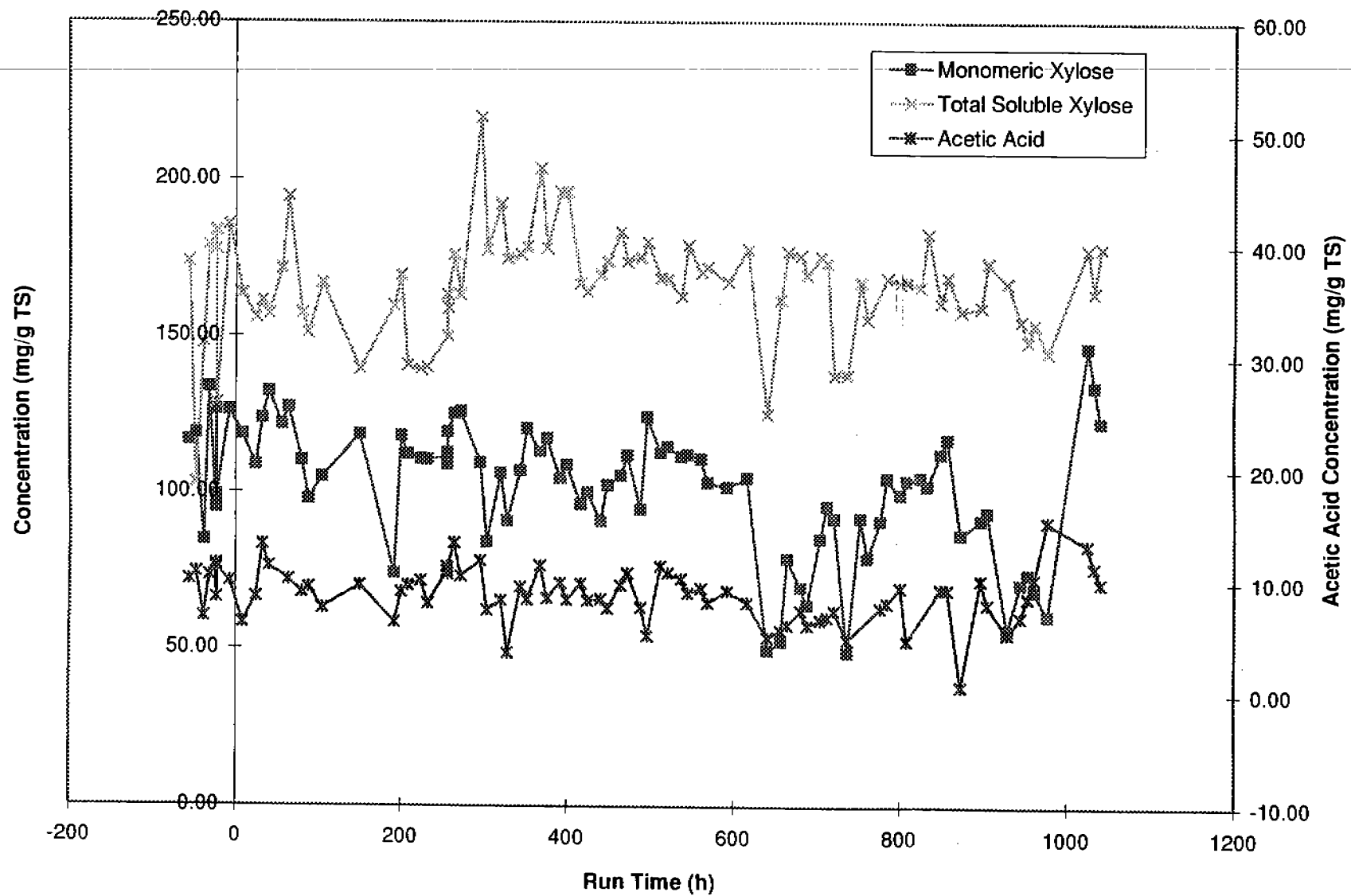


Figure 5. APR Glucose and Xylose Yields

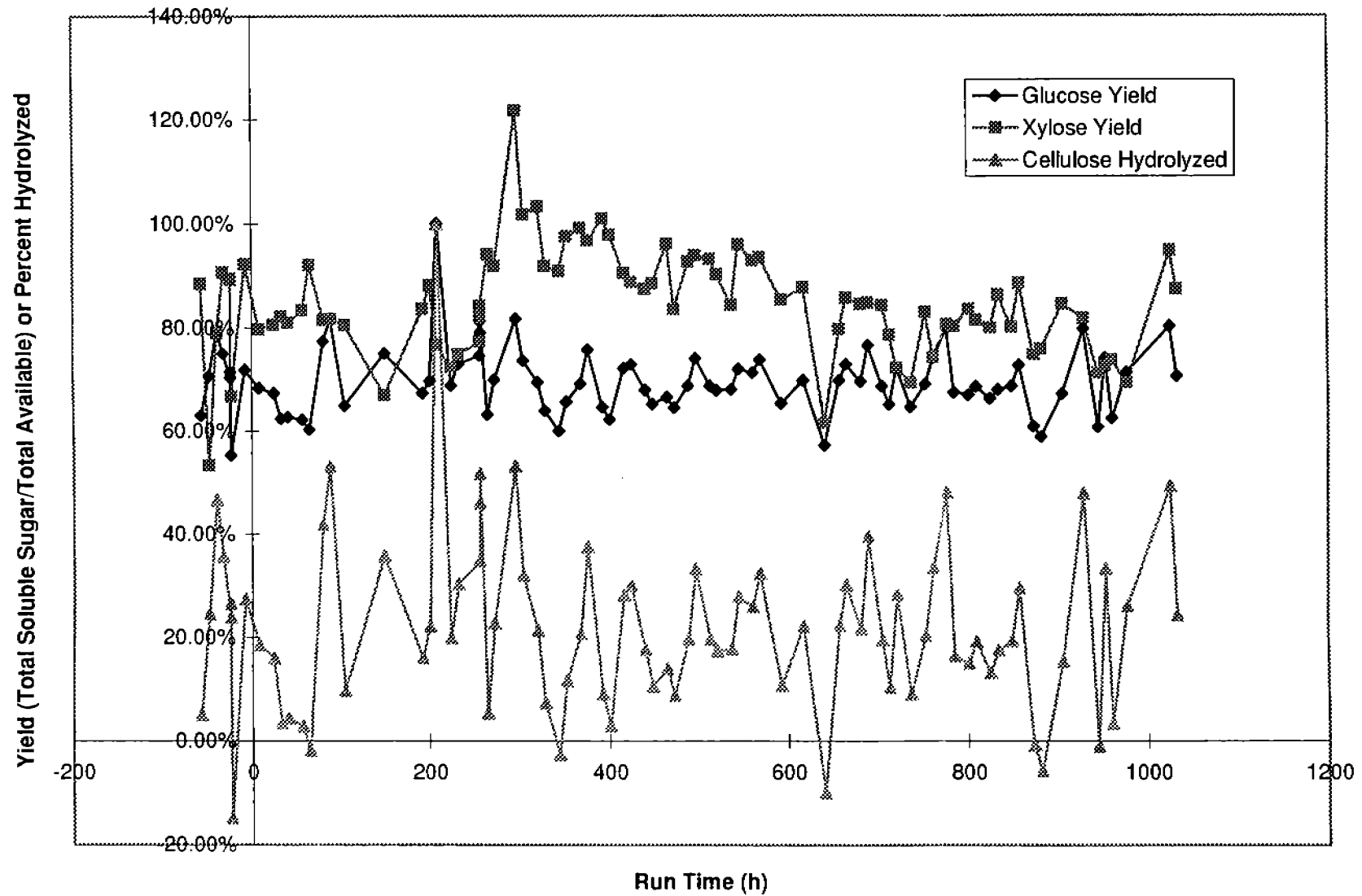
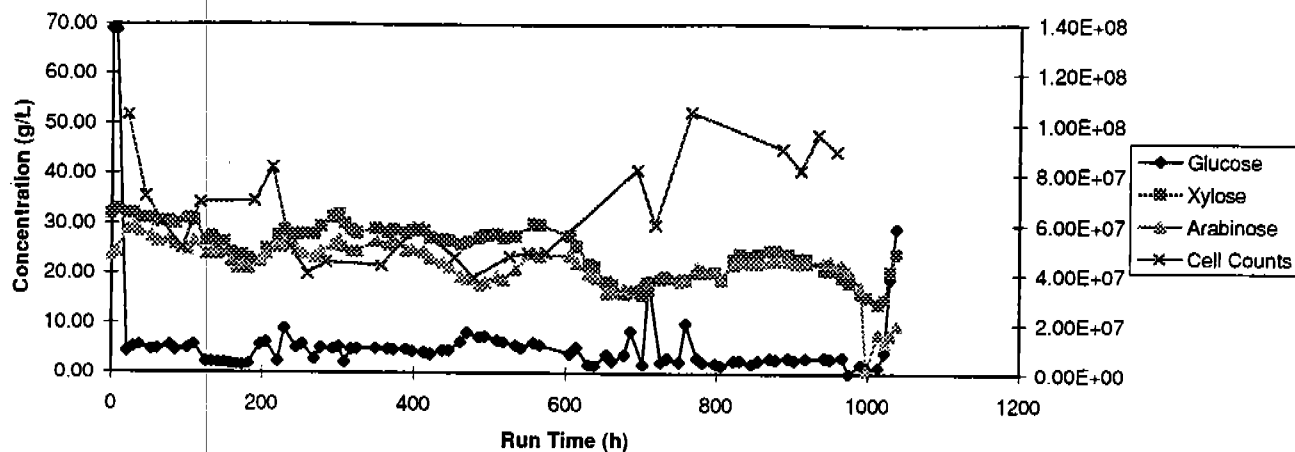
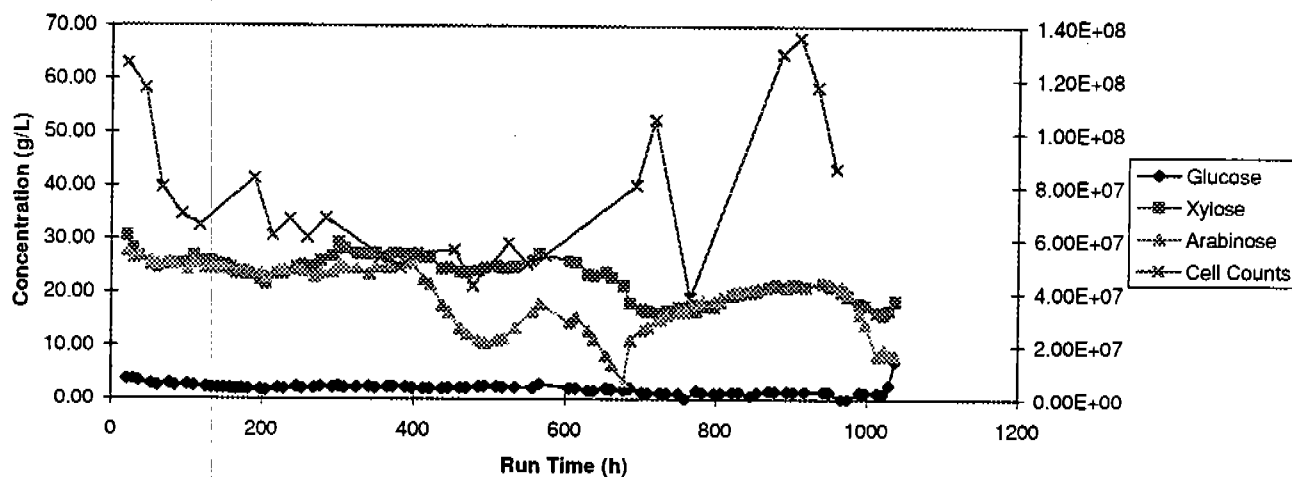


Figure 8. Monomeric Sugar Concentrations and Cell Counts in the SSCF Fermenters

### First 9000-L Fermenter Monomeric Sugar Concentrations and Cell Counts



### Second 9000-L Fermenter Monomeric Sugar Concentrations and Cell Counts



### Third 9000-L Fermenter Monomeric Sugar Concentrations and Cell Counts

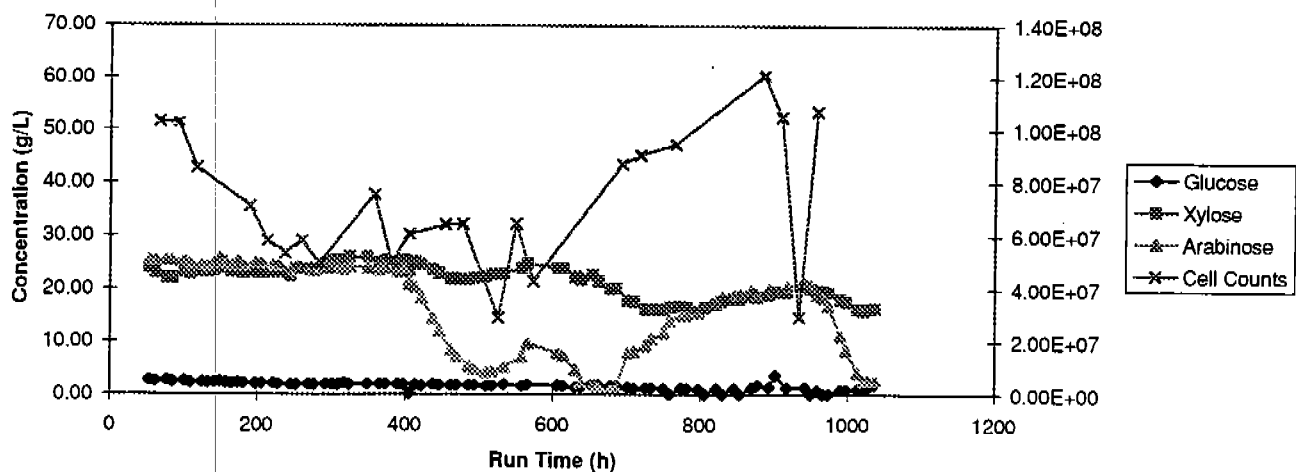
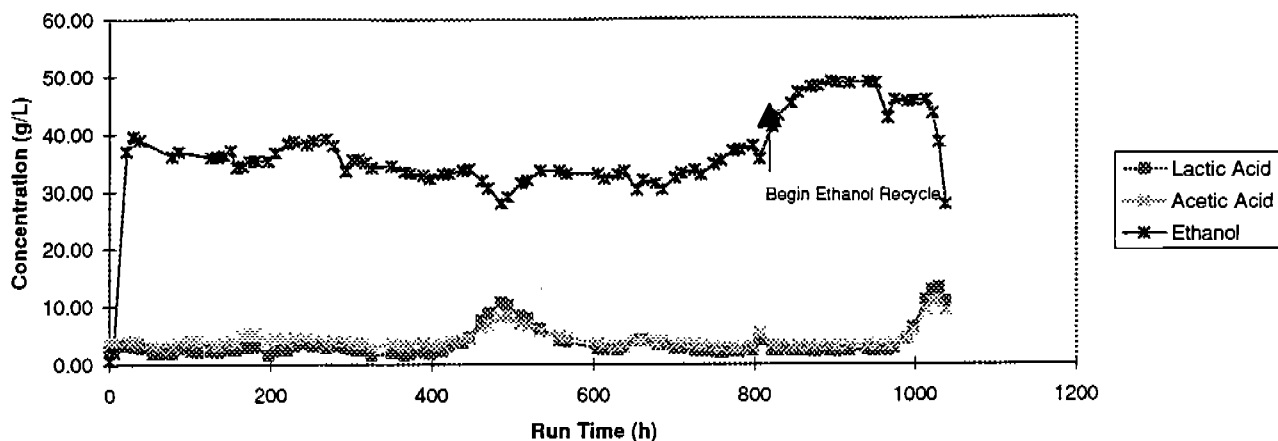
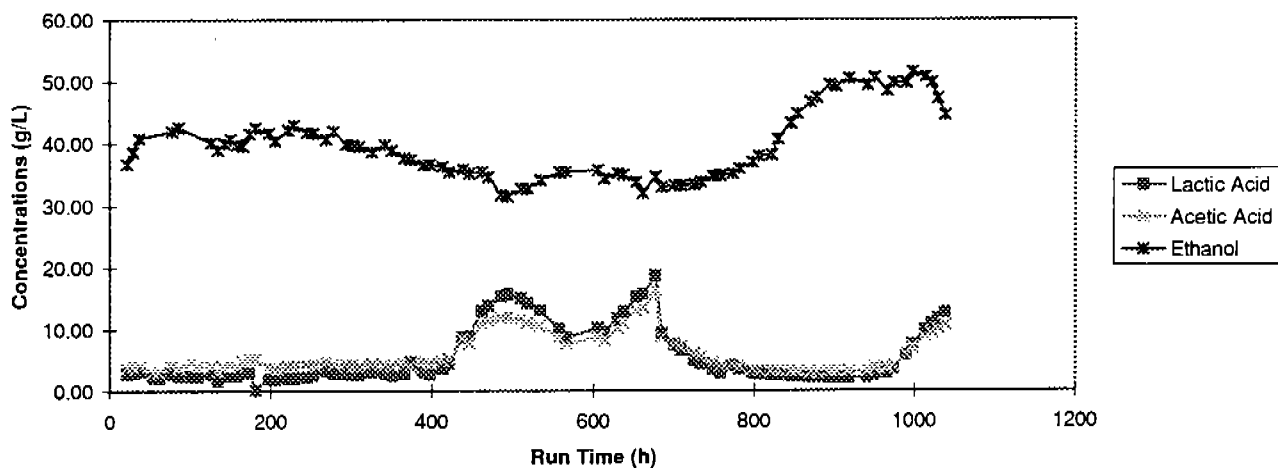


Figure 9. Product Concentrations in the SSCF Fermenters

### First 9000-L Fermenter Product Concentrations



### Second 9000-L Fermenter Product Concentrations



### Third 9000-L Fermenter Product Concentrations

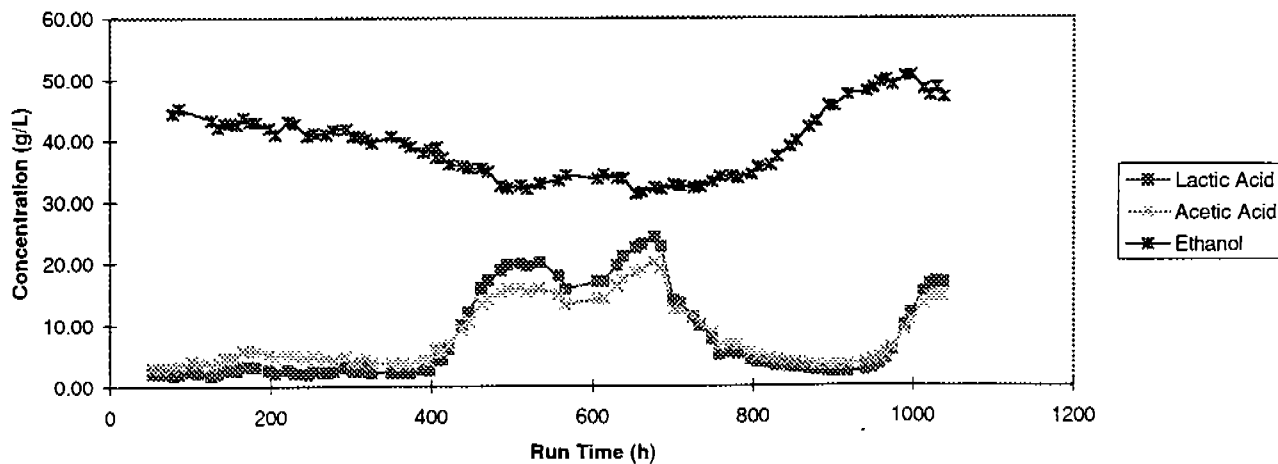
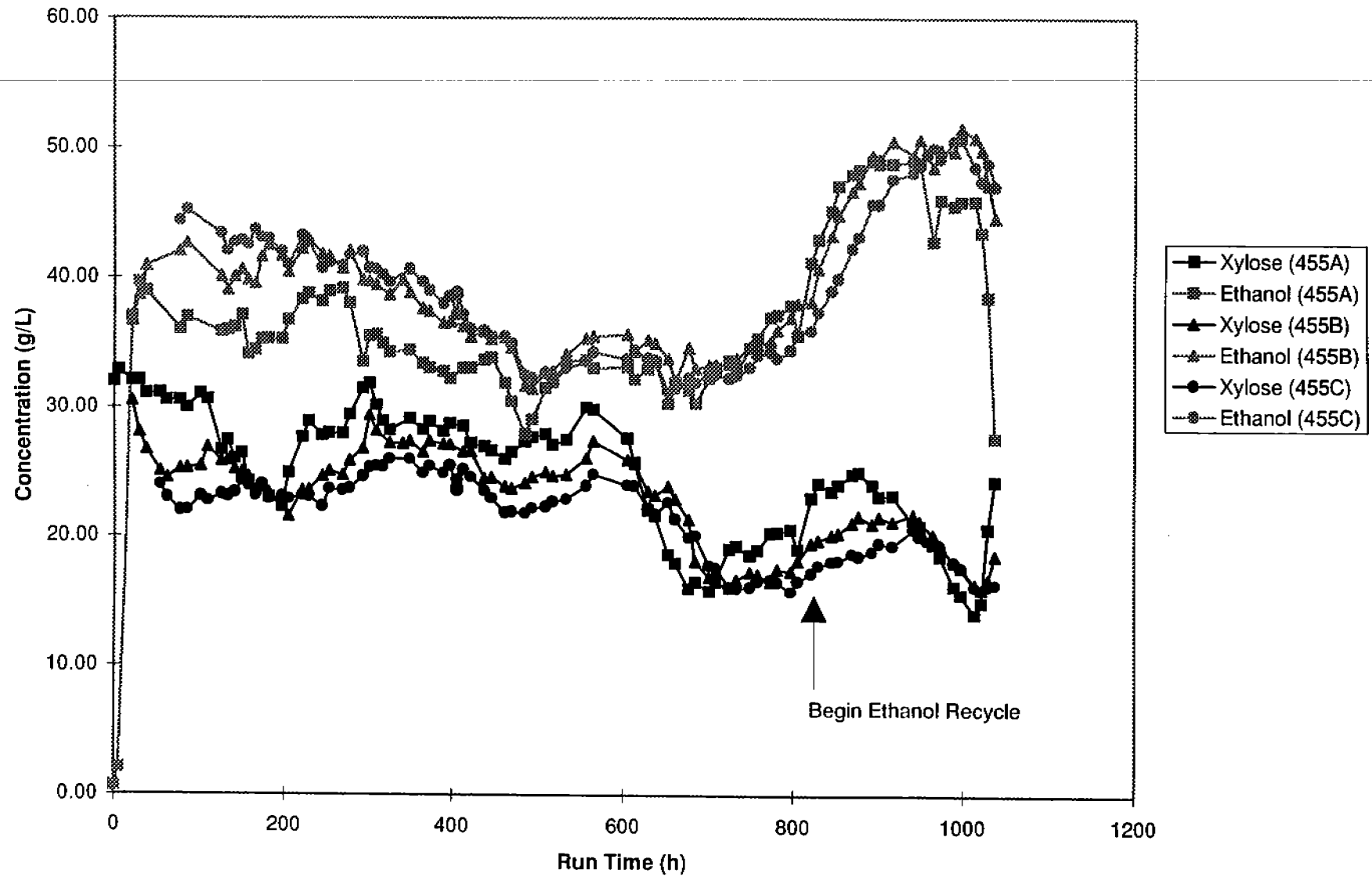
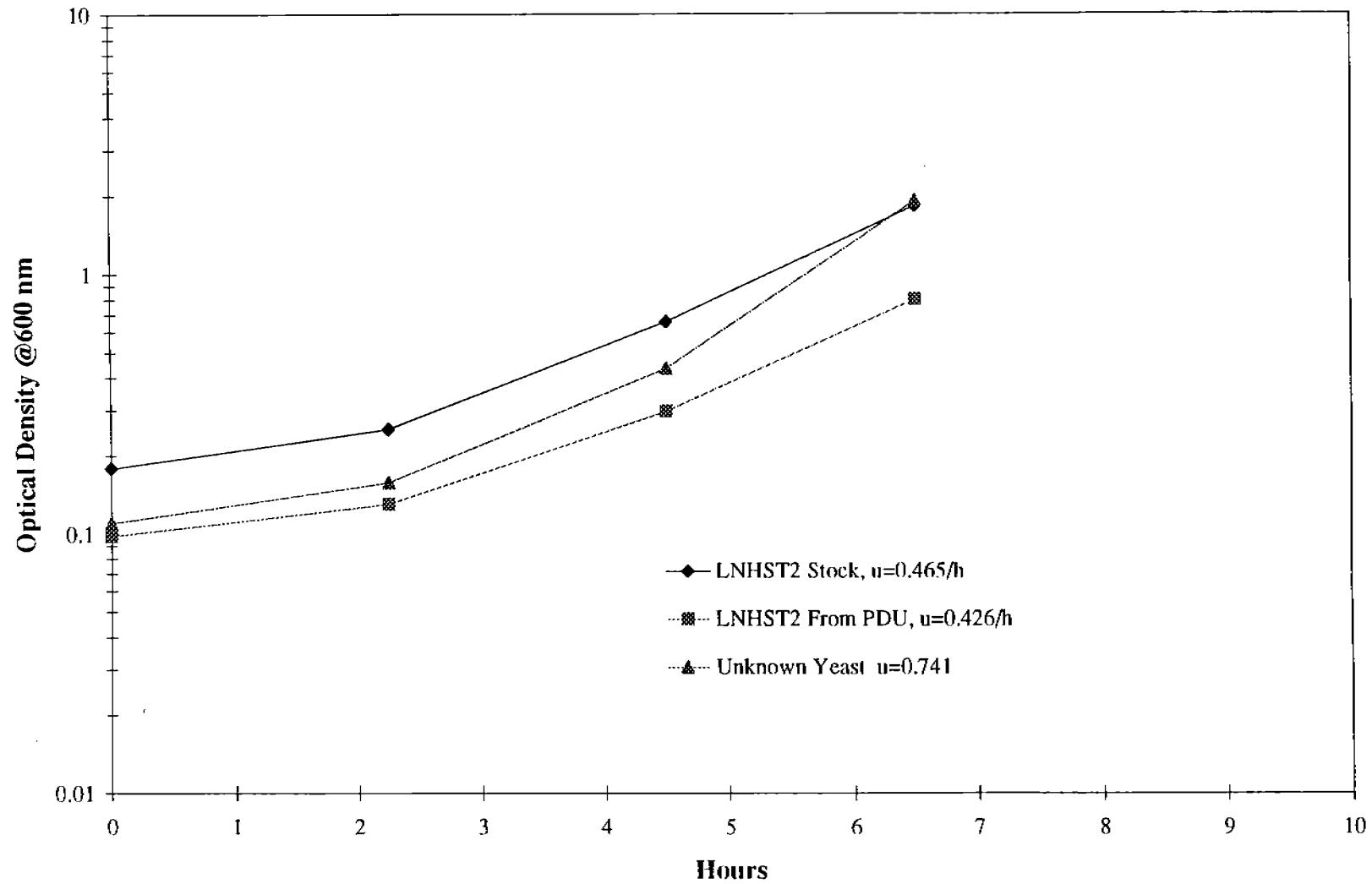


Figure 10. Ethanol and Xylose in the 9000-L Fermenters



**Figure 11. Growth rate of LNHST2 From Stock and PDU run and an unknown yeast from the same run.**



**Figure 12. Comparison of fermentation products and xylose consumption between LNHST2 from Stock, LNHST2 from PDU run and an unknown yeast from the same run.**

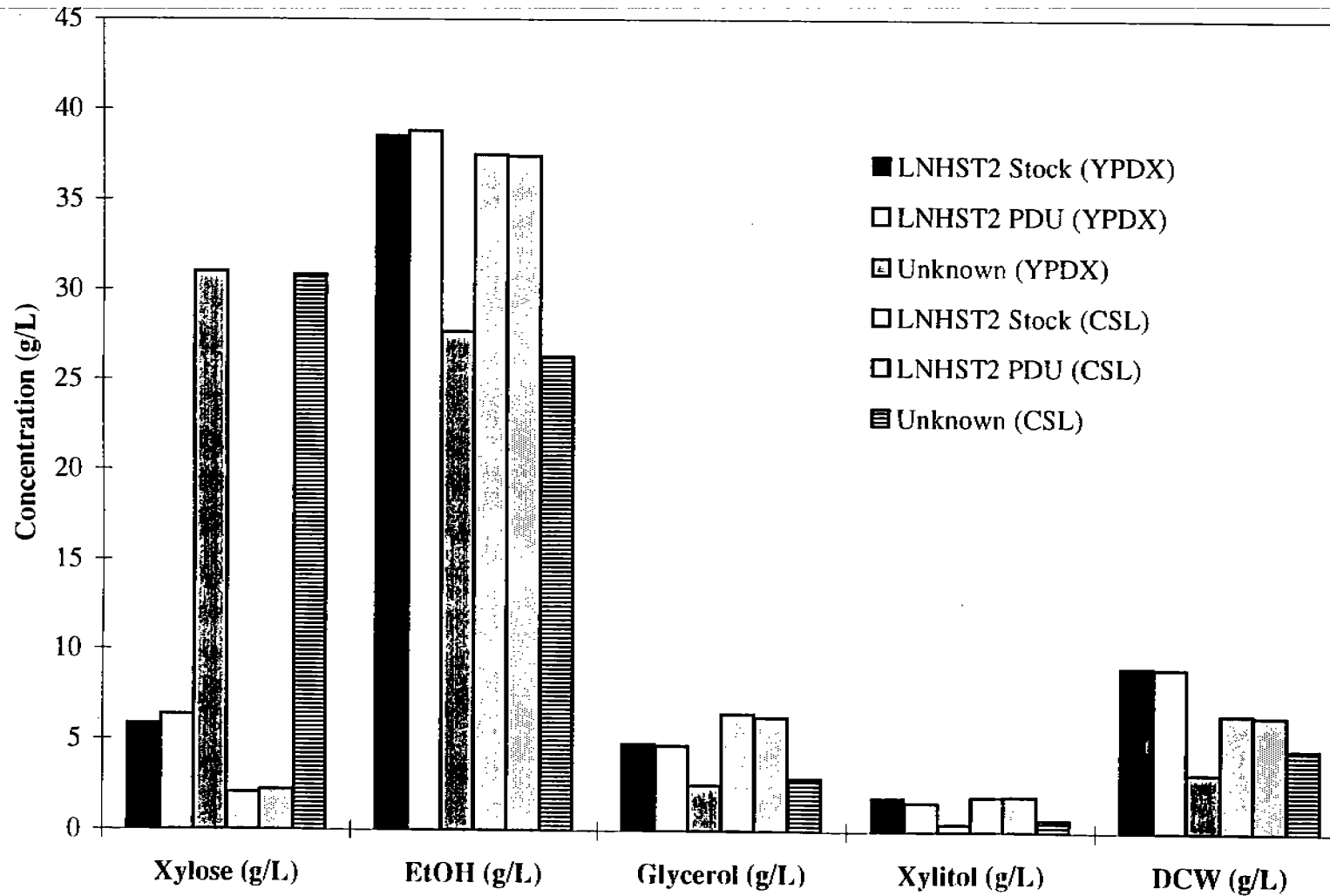


Figure 13. Ethanol Concentrations at Steady State

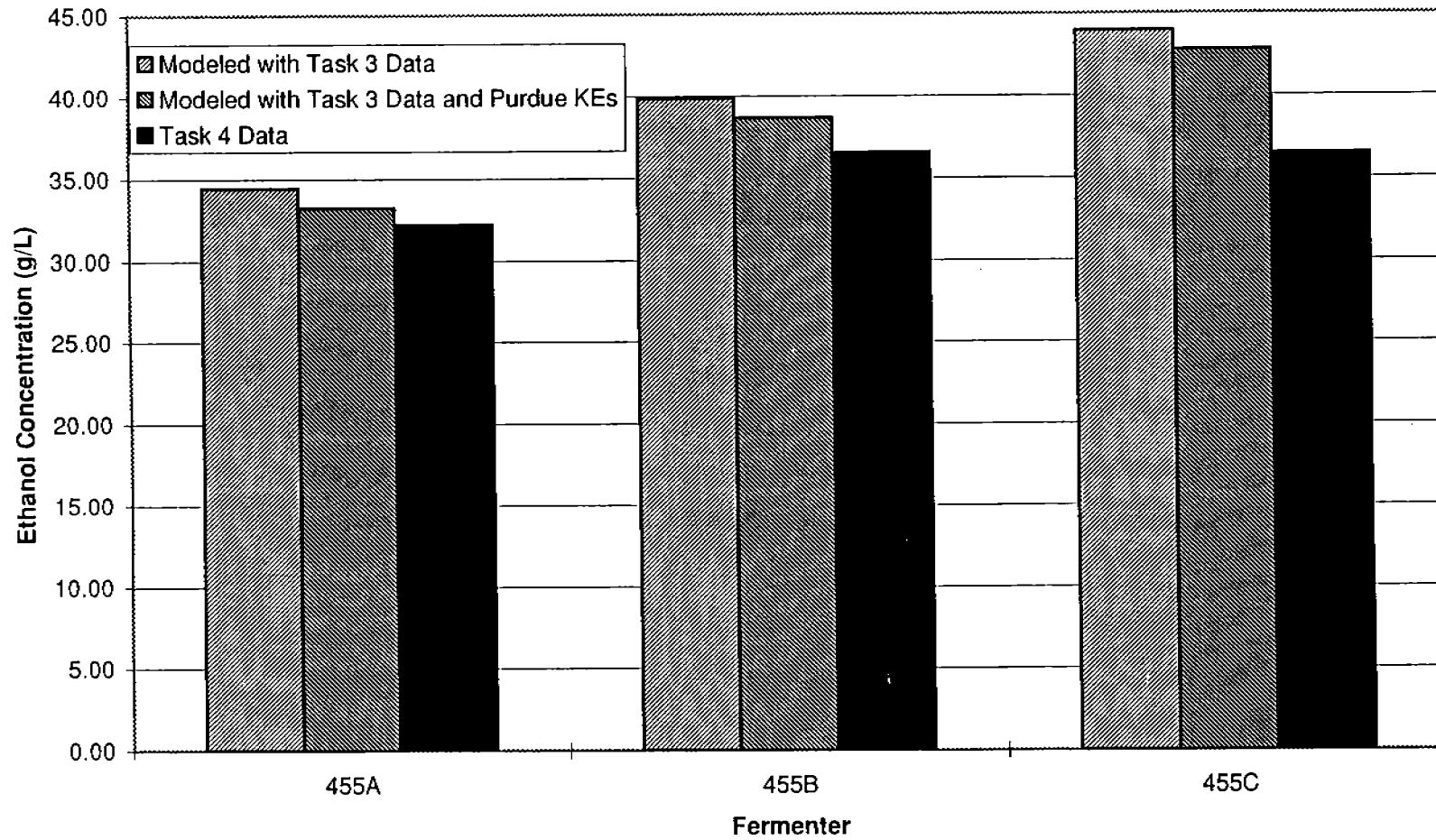


Figure 14. Xylose Concentrations at Steady State

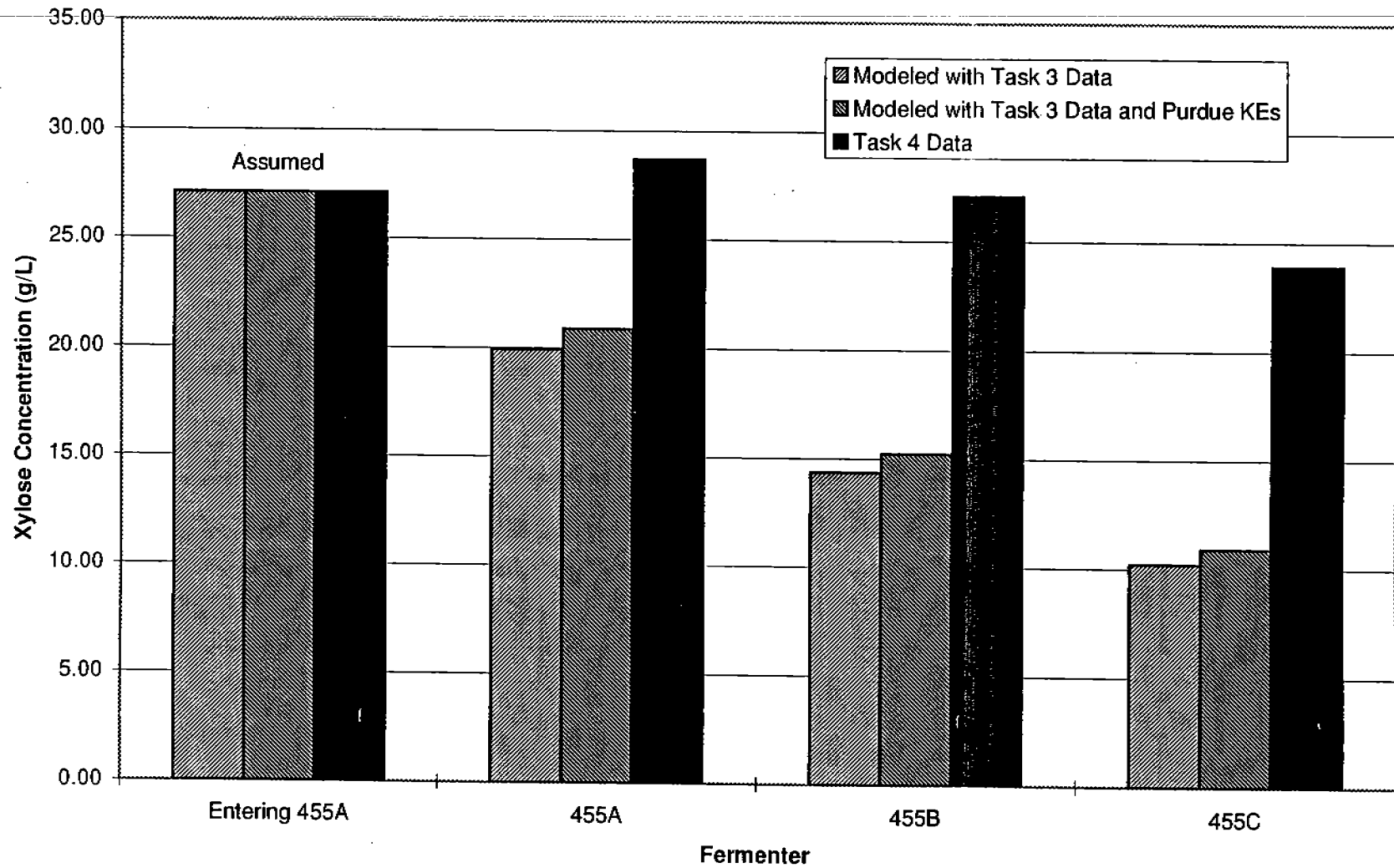


Figure 15. Glucose Concentrations at Steady State

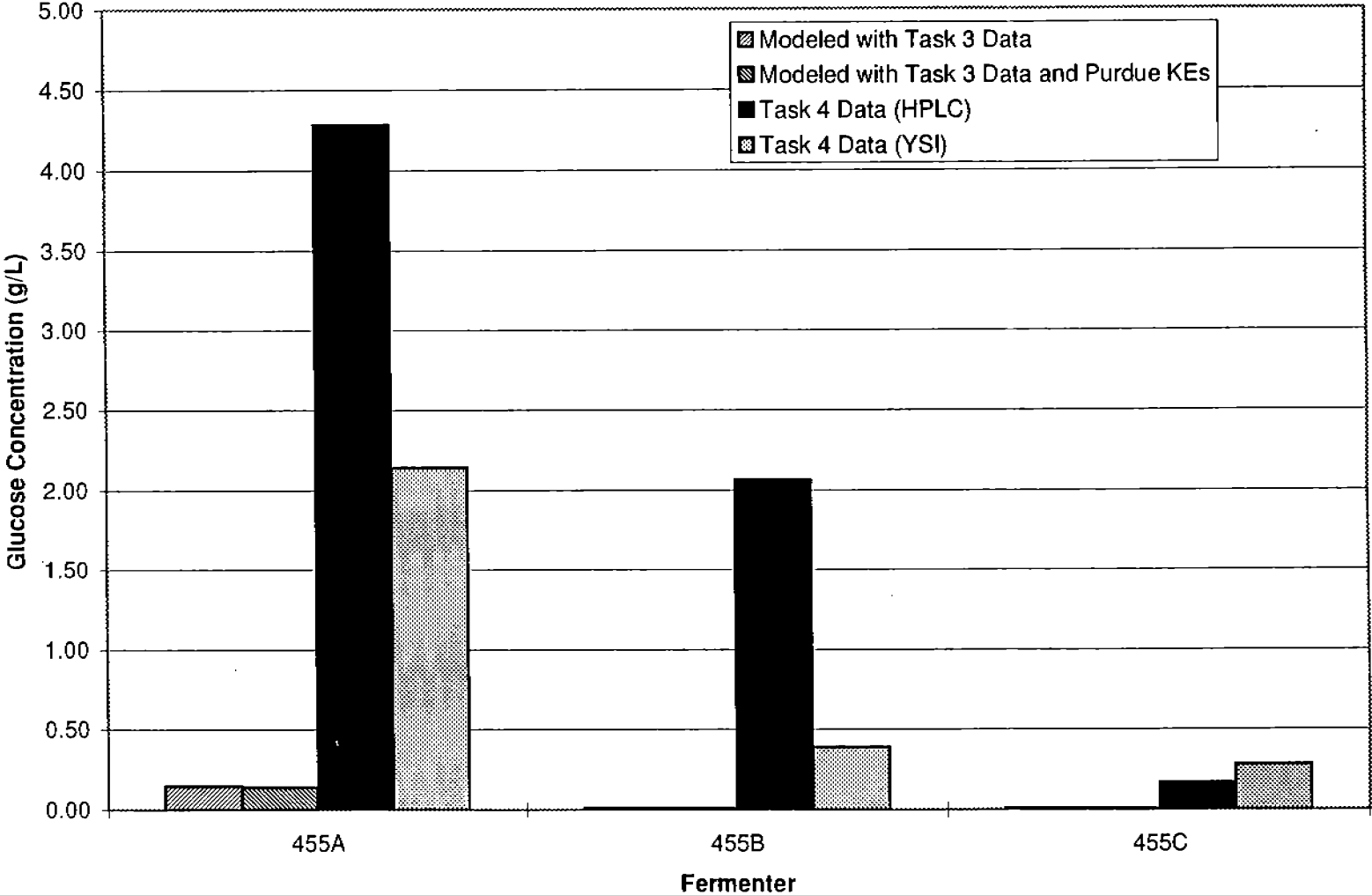
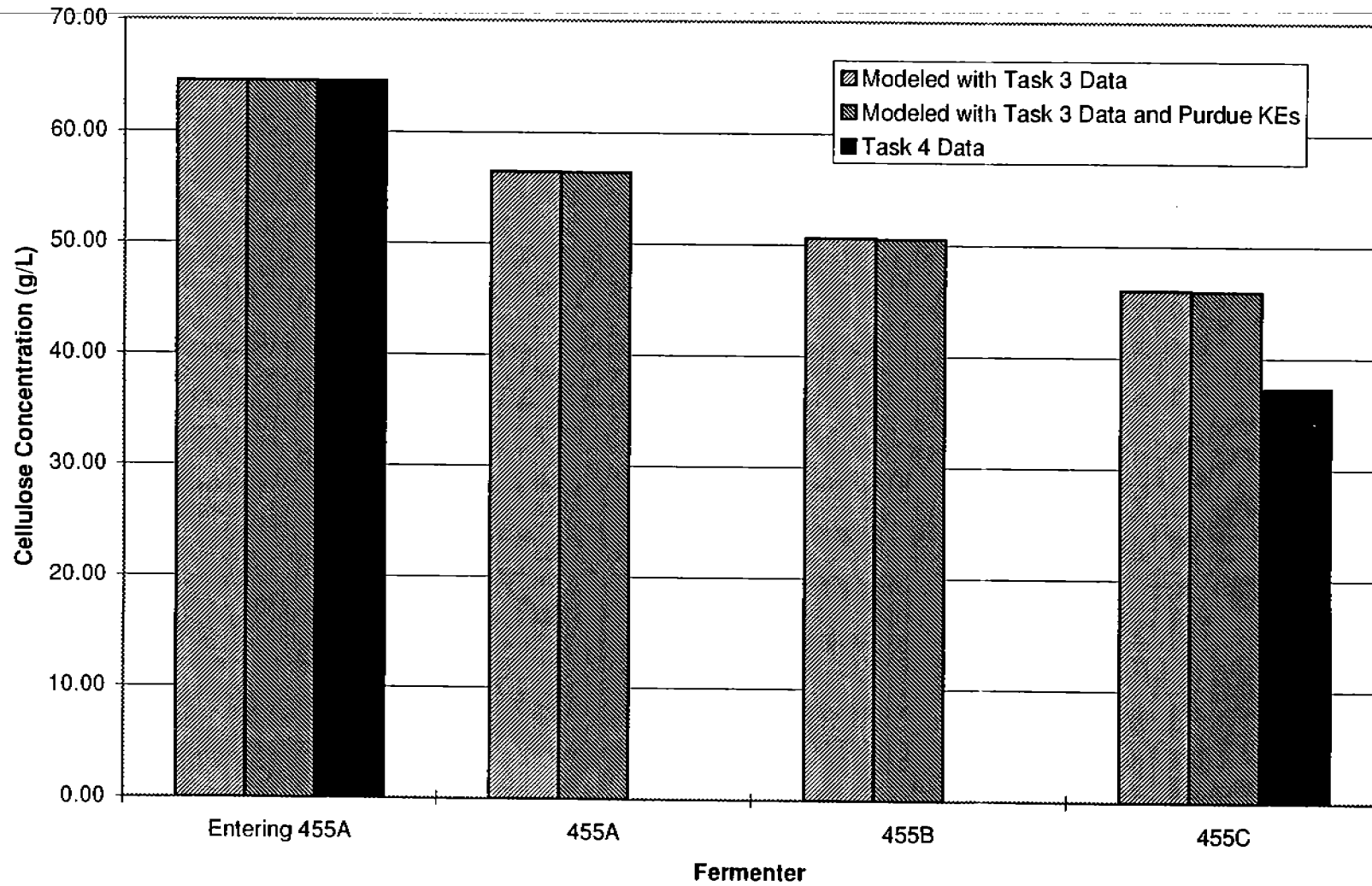


Figure 16. Cellulose Concentrations at Steady State



Run start date 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

# APR Data

Date	Time	Run time (h)	APR #	Tot. Solids Oven (%)	TDS Liquid (%)	Ins.Solids (%)	Sample Wt. (g)	HPLC (g/L)								
								Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic
3/15/96	4:00	-56.5	210	35.85%	0.00%	9.07%	25.08	77.77	45.89	11.28	37.44	0.00	0.00	1.93	0.00	2.36
3/15/96	13:00	-47.5	211	37.55%	0.00%	9.50%	25.05	94.03	49.24	11.35	34.47	11.07	0.00	2.67	0.00	2.11
3/15/96	22:00	-38.5	212	38.23%	0.00%	9.67%	25.05	67.39	35.83	8.92	32.63	7.52	0.00	0.25	0.00	0.00
3/16/96	5:00	-31.5	213	36.39%	0.00%	9.21%	25.07	102.72	53.46	11.77	35.06	11.20	0.00	1.79	0.00	0.67
3/16/96	13:00	-23.5	214A	35.60%	0.00%	8.39%	25.05	69.35	36.94	7.90	30.54	0.00	0.00	1.68	0.00	1.19
3/16/96	13:05	-23.33333	214B	34.31%	0.00%	7.89%	25.10	86.99	47.05	9.45	32.14	0.00	0.00	2.92	0.00	0.00
3/16/96	13:20	-23.16667	214C	36.61%	0.00%	8.58%	25.22	74.19	39.60	8.03	32.40	0.00	0.00	0.66	0.00	0.00
3/17/96	5:00	-7.5	216	33.63%	0.00%	8.41%	25.25	83.90	46.31	10.05	33.56	8.69	0.00	2.17	0.00	1.90
3/17/96	21:00	8.5	218	32.11%	0.00%	8.03%	25.63	78.30	41.30	8.63	29.69	7.56	0.00	0.00	0.00	0.00
3/18/96	13:00	24.5	220	35.76%	0.00%	8.94%	25.22	80.08	42.68	10.39	33.23	0.00	0.00	2.44	0.00	2.34
3/18/96	21:00	32.5	221	27.53%	0.00%	6.88%	25.27	63.77	36.51	8.37	25.88	0.00	0.00	0.00	0.00	0.37
3/19/96	5:00	40.5	222	26.75%	0.00%	6.69%	26.02	66.12	37.86	9.30	26.79	0.00	0.00	1.97	0.00	1.49
3/19/96	21:00	56.5	224	25.43%	0.00%	6.36%	27.16	58.44	33.00	7.84	24.60	5.05	0.00	0.00	0.00	0.94
3/20/96	5:00	64.5	228	25.06%	0.00%	6.27%	25.15	52.20	33.97	8.39	26.02	0.00	0.00	0.42	0.00	0.00
3/20/96	21:00	80.5	227	28.40%	0.00%	7.10%	28.96	76.63	33.64	7.93	23.67	0.00	0.00	2.10	0.00	1.93
3/21/96	5:00	88.5	231	28.44%	0.00%	7.11%	27.37	72.47	30.00	8.69	24.29	0.00	0.00	0.00	0.00	1.13
3/21/96	21:00	104.5	230	25.97%	0.00%	6.49%	28.09	50.02	29.14	7.97	23.95	0.00	0.00	0.00	0.00	0.58
3/23/96	18:40	150.16667	232	27.66%	0.00%	6.92%	25.43	82.20	35.18	8.90	23.03	0.00	0.00	1.82	0.00	0.00
3/25/96	13:00	192.5	233	32.22%	0.00%	8.05%	27.07	38.99	25.96	7.34	30.86	2.63	0.00	1.35	0.00	3.28
3/25/96	21:00	200.5	234	32.50%	0.00%	8.12%	28.04	74.43	41.76	9.90	32.17	6.87	0.00	1.05	0.00	1.79
3/26/96	5:00	208.5	235	36.56%	0.00%	9.14%	24.96	137.40	45.15	9.25	27.38	9.35	0.00	2.17	0.00	1.04
3/26/96	21:00	224.5	237	33.91%	0.00%	8.48%	26.45	87.91	40.99	9.34	28.85	0.00	0.00	2.28	0.00	3.83
3/27/96	5:00	232.5	238	34.43%	0.00%	8.61%	25.78	88.07	41.63	9.95	30.48	0.00	0.00	0.87	0.00	1.51
3/28/96	5:00	256.5	240	34.08%	0.00%	8.52%	25.41	95.18	41.51	8.82	27.32	0.00	0.00	2.49	0.00	4.12
3/28/96	5:10	256.66667	240	34.08%	0.00%	8.52%	5.00	94.25	40.60	9.61	24.38	0.00	0.00	0.88	0.00	1.50
3/28/96	5:20	256.83333	240	34.08%	0.00%	8.52%	8.26	94.26	41.96	9.46	26.55	0.00	0.00	1.91	0.00	3.25
3/28/96	5:30	257	240	34.08%	0.00%	8.52%	25.20	100.85	44.45	10.61	28.83	0.00	0.00	1.76	0.00	3.08
3/28/96	13:40	265.16667	241	34.97%	0.00%	8.74%	25.69	73.50	47.94	11.03	35.03	0.00	0.00	1.89	0.00	2.27
3/28/96	21:00	272.5	242	37.20%	0.00%	9.30%	25.51	90.72	51.59	11.36	34.67	0.00	0.00	0.95	0.00	0.00
3/29/96	21:00	296.5	245	36.59%	0.00%	9.15%	25.10	71.73	44.05	10.82	34.14	0.00	0.00	0.00	0.00	2.15
3/30/96	5:00	304.5	246	36.82%	0.00%	9.21%	25.12	48.84	34.14	9.15	33.49	0.00	0.00	0.00	0.00	0.00
3/30/96	21:00	320.5	248	35.63%	0.00%	8.91%	25.15	59.21	41.48	10.40	34.36	0.00	0.00	1.59	0.00	1.71
3/31/96	5:00	328.5	249	35.78%	0.00%	8.95%	25.07	48.69	35.76	9.41	33.67	0.00	0.00	0.47	0.00	0.17
3/31/96	21:00	344.5	251	34.27%	0.00%	8.57%	25.03	55.76	40.09	10.13	33.87	6.43	0.00	1.53	0.00	2.07
4/1/96	5:00	352.5	252	35.63%	0.00%	8.91%	27.87	70.47	47.14	11.67	35.90	7.91	0.00	0.20	0.00	0.00
4/1/96	21:00	368.5	254	32.73%	0.00%	8.18%	25.70	66.12	40.33	10.53	31.12	2.40	0.00	15.21	0.00	1.82
4/2/96	5:00	376.5	255	37.03%	0.00%	9.26%	29.10	83.86	47.90	12.42	35.55	0.00	0.00	0.51	0.00	0.00
4/2/96	21:00	392.5	257	35.33%	0.00%	8.83%	25.17	57.97	40.54	10.31	33.88	6.62	0.00	1.70	0.00	3.48
4/3/96	5:00	400.5	258	33.56%	0.00%	8.39%	26.18	54.61	39.83	10.25	33.68	6.67	0.00	0.00	0.00	0.00

Run start data 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

Date	Time	Run time (h)	APR #						Liquor Analysis (Total Sugars)				
				Glycerol	Acetic	EtOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
3/15/96	4:00	-56.5	210	0.00	4.00	0.00	0.00	0.00	118.34	68.50	13.76	42.07	0.00
3/15/96	13:00	-47.5	211	0.00	4.51	0.00	0.00	0.00	137.60	42.82	14.07	43.32	0.00
3/15/96	22:00	-38.5	212	0.00	2.91	0.00	0.00	0.00	151.77	62.48	13.24	42.99	0.00
3/16/96	5:00	-31.5	213	0.00	4.22	0.00	0.00	0.00	143.72	71.69	14.86	44.65	0.00
3/16/96	13:00	-23.5	214A	0.00	4.49	0.00	0.00	0.00	131.26	68.87	11.60	43.49	0.00
3/16/96	13:05	-23.33333	214B	0.00	3.17	0.00	0.00	0.00	132.51	68.30	12.45	43.26	0.00
3/16/96	13:20	-23.16667	214C	0.00	4.52	0.00	0.00	0.00	103.34	51.42	12.30	37.19	0.00
3/17/96	5:00	-7.5	216	0.00	3.68	0.00	0.00	0.00	128.53	68.14	16.44	42.57	10.88
3/17/96	21:00	8.5	218	0.00	2.20	0.00	0.00	0.00	118.44	57.01	11.07	34.38	11.36
3/18/96	13:00	24.5	220	0.00	3.39	0.00	0.00	0.00	123.89	61.19	13.03	36.96	10.35
3/18/96	21:00	32.5	221	0.00	3.95	0.00	0.00	0.00	87.52	47.60	10.25	28.27	7.85
3/19/96	5:00	40.5	222	0.00	3.26	0.00	0.00	0.00	84.51	45.00	9.59	26.67	7.59
3/19/96	21:00	56.5	224	0.00	0.00	0.00	0.00	0.00	84.13	46.54	12.04	29.61	0.00
3/20/96	5:00	64.5	228	0.00	2.73	0.00	0.00	0.00	82.59	51.98	13.28	33.07	11.33
3/20/96	21:00	80.5	227	0.00	2.77	0.00	0.00	0.00	110.68	48.09	12.30	30.15	12.71
3/21/96	5:00	88.5	231	0.30	2.91	0.00	0.10	0.94	112.09	46.19	11.39	28.17	13.74
3/21/96	21:00	104.5	230	0.10	2.12	0.00	0.00	0.87	90.46	46.34	11.32	28.98	12.48
3/23/96	18:40	150.16667	232	0.00	2.87	0.00	0.00	0.00	112.57	41.44	9.55	26.88	11.04
3/25/96	13:00	192.5	233	0.00	2.23	0.00	0.00	0.00	109.10	55.94	12.35	37.57	0.00
3/25/96	21:00	200.5	234	0.00	3.21	0.00	0.00	0.00	114.85	59.91	13.00	38.13	12.80
3/26/96	5:00	208.5	235	0.00	3.89	0.00	0.57	0.00	178.84	56.54	13.09	34.38	0.00
3/26/96	21:00	224.5	237	0.00	3.74	0.00	0.00	0.00	118.87	51.55	10.86	31.60	11.58
3/27/96	5:00	232.5	238	0.00	3.02	2.68	0.00	0.00	124.62	52.79	10.75	32.34	11.27
3/28/96	5:00	256.5	240	0.00	4.08	0.00	0.00	0.00	130.56	55.82	12.48	34.61	0.00
3/28/96	5:10	256.66667	240	0.00	4.23	0.00	0.00	0.00	138.15	60.66	16.56	32.53	0.00
3/28/96	5:20	256.83333	240	0.00	3.97	0.00	0.00	0.00	138.32	59.19	15.06	35.22	0.00
3/28/96	5:30	257	240	0.00	4.21	0.00	0.00	0.00	142.07	60.78	15.63	36.69	0.00
3/28/96	13:40	265.16667	241	0.00	5.16	0.00	0.00	0.00	109.68	67.37	15.65	43.61	0.00
3/28/96	21:00	272.5	242	0.00	4.30	0.00	0.00	0.00	123.57	66.93	15.39	42.82	0.00
3/29/96	21:00	296.5	245	0.00	4.79	0.00	0.00	0.00	143.98	88.52	31.93	57.62	27.69
3/30/96	5:00	304.5	246	0.00	3.02	0.00	0.00	0.00	126.11	71.92	19.98	45.71	9.90
3/30/96	21:00	320.5	248	0.00	3.26	0.00	0.00	0.00	122.50	75.18	18.32	46.08	7.28
3/31/96	5:00	328.5	249	0.00	1.43	0.00	0.00	0.00	115.78	68.61	16.56	42.81	14.05
3/31/96	21:00	344.5	251	0.00	3.58	0.00	0.00	0.00	105.56	65.93	14.35	42.64	11.79
4/1/96	5:00	352.5	252	0.00	3.29	0.00	0.00	0.00	113.76	69.76	14.98	44.35	12.57
4/1/96	21:00	368.5	254	0.00	4.10	0.00	0.00	0.00	122.50	72.50	20.13	49.14	19.42
4/2/96	5:00	376.5	255	0.00	3.49	0.00	0.00	0.00	137.81	72.64	15.52	45.52	15.54
4/2/96	21:00	392.5	257	0.00	3.83	0.00	0.00	0.00	117.69	75.90	19.16	50.97	7.42
4/3/96	5:00	400.5	258	0.00	3.08	0.00	0.00	0.00	110.48	71.70	16.29	47.27	6.99

Run start data 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

Date	Time	Run time (h)	APR #	Tot. Solids Oven (%)	TDS Liquid (%)	Ins. Solids (%)	Sample Wt. (g)	HPLC (g/L)								
								Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic
4/3/96	21:00	416.5	260	35.21%	0.00%	8.80%	25.94	62.72	37.21	10.01	30.51	0.00	0.00	1.45	0.00	2.10
4/4/96	5:00	424.5	261	35.42%	0.00%	8.85%	27.99	70.51	38.89	10.15	30.25	0.00	0.00	0.00	0.00	0.00
4/4/96	21:00	440.5	263	33.85%	0.00%	8.46%	27.39	54.77	33.65	9.67	31.14	2.14	0.00	1.30	0.00	2.13
4/5/96	5:00	448.5	264	35.49%	0.00%	8.87%	27.35	64.59	39.93	10.56	33.43	2.57	0.00	0.16	0.00	0.00
4/5/96	21:00	464.5	266	35.02%	0.00%	8.76%	25.05	60.69	40.46	10.43	33.98	2.18	0.00	1.82	0.00	2.26
4/6/96	5:00	472.5	267	35.35%	0.00%	8.84%	25.10	76.11	43.36	10.23	32.39	6.95	0.00	1.27	0.00	0.00
4/6/96	21:00	488.5	269	35.39%	0.00%	8.85%	25.09	55.84	36.75	10.22	32.99	2.72	0.00	1.46	0.00	1.82
4/7/96	5:00	496.5	270	36.27%	0.00%	9.07%	25.14	90.89	49.53	12.01	34.40	8.95	0.00	1.37	0.00	0.00
4/7/96	21:00	512.5	272	35.51%	0.00%	8.88%	25.04	68.21	43.99	11.40	34.06	4.25	0.00	1.93	0.00	2.57
4/8/96	5:00	520.5	273	36.79%	0.00%	9.20%	25.26	75.51	46.52	11.81	34.22	4.20	0.00	1.16	0.00	0.00
4/8/96	21:00	536.5	275	36.74%	0.00%	9.18%	25.23	81.43	45.14	11.00	32.28	3.04	0.00	2.08	0.00	2.08
4/9/96	5:00	544.5	276	35.81%	0.00%	8.95%	25.41	71.24	44.12	11.71	35.16	3.18	0.00	1.35	0.00	0.00
4/9/96	21:00	560.5	278	35.89%	0.00%	8.97%	25.48	75.48	43.72	10.33	32.63	2.91	0.00	2.05	0.00	0.00
4/10/96	5:00	568.5	279	35.59%	0.00%	8.90%	25.05	70.41	40.34	10.00	32.31	2.52	0.00	0.95	0.00	0.00
4/11/96	5:00	592.5	282	35.28%	0.00%	8.82%	25.52	64.86	39.39	10.07	31.52	2.93	0.00	1.55	0.00	0.00
4/12/96	5:00	616.5	285	33.29%	0.00%	8.32%	25.31	65.95	38.03	9.92	30.73	6.13	0.00	0.99	0.00	0.00
4/13/96	5:00	640.5	286	33.63%	0.00%	8.41%	33.63	28.15	18.27	5.87	21.37	3.78	0.00	0.83	0.00	2.10
4/13/96	21:00	656.5	288	31.97%	0.00%	7.99%	25.35	23.17	18.28	6.22	26.81	3.27	0.00	1.05	0.00	1.61
4/14/96	5:00	664.5	289	32.02%	0.00%	8.01%	25.70	41.61	27.58	8.97	29.83	3.06	0.00	1.07	0.00	1.38
4/14/96	21:00	680.5	291	31.61%	0.00%	7.90%	25.77	33.87	23.95	7.75	28.16	2.70	0.00	1.08	0.00	2.99
4/15/96	5:00	688.5	292	33.21%	0.00%	8.30%	25.72	35.16	23.29	7.64	28.04	2.88	0.00	0.83	0.00	0.00
4/15/96	21:00	704.5	293	32.87%	0.00%	8.22%	28.23	50.77	30.63	8.28	28.88	4.54	0.00	1.13	0.00	1.44
4/16/96	5:00	712.5	294	33.25%	0.00%	8.31%	28.19	63.46	34.76	8.79	29.02	0.00	0.00	0.76	0.00	0.51
4/16/96	13:20	720.8	295	36.42%	0.00%	8.22%	27.61	81.76	36.52	4.01	25.49	0.00	0.00	1.55	0.00	0.87
4/17/96	5:00	736.5	296	33.49%	0.00%	8.37%	27.65	24.17	17.91	5.10	25.34	0.00	0.00	0.88	0.00	1.56
4/17/96	21:00	752.5	299	33.33%	0.00%	8.33%	26.85	59.78	33.48	8.70	28.89	0.00	0.00	1.35	0.00	2.76
4/18/96	5:30	761	300	34.61%	0.00%	8.65%	27.60	52.01	30.05	8.11	27.99	2.31	0.00	0.44	0.00	0.76
4/18/96	21:00	776.5	302	36.51%	0.00%	9.13%	24.96	78.24	36.63	9.70	28.73	0.00	0.00	1.90	0.00	2.05
4/19/96	5:00	784.5	303	33.32%	0.00%	8.33%	25.04	69.62	38.09	10.04	30.66	0.00	0.00	0.86	0.00	0.94
4/19/96	21:00	800.5	305	35.27%	0.00%	8.82%	25.11	68.61	38.53	9.81	30.73	3.09	0.00	2.43	0.00	2.43
4/20/96	5:00	808.5	306	34.73%	0.00%	8.68%	25.25	75.76	39.55	10.02	30.37	2.97	0.00	0.97	0.00	0.00
4/20/96	21:00	824.5	308	35.08%	0.00%	8.77%	25.23	68.45	40.32	10.18	32.53	0.00	0.00	1.61	0.00	1.66
4/21/96	5:30	833	309	35.22%	0	8.80%	25.67	72.97	39.52	10.14	31.03	0.00	0.00	1.27	0.00	1.77
4/21/96	21:00	848.5	311	36.41%	0	9.10%	26.15	90.19	45.05	9.85	30.75	0.00	0.00	2.42	0.00	2.74
4/22/96	5:00	856.5	312	36.61%	0	9.15%	26.25	89.49	47.26	10.29	31.40	0.00	0.00	1.82	0.00	0.00
4/22/96	21:00	872.5	314	35.12%	0	8.78%	25.06	55.65	33.40	9.08	31.05	0.00	0.00	1.38	0.00	0.00
4/23/96	5:00	880.5	315	34.45%	0	8.61%	29.21	48.94	29.67	8.41	27.57	2.11	0.00	9.73	0.00	0.00
4/23/96	21:00	896.5	317	35.37%	0	8.84%	27.30	59.33	35.49	9.59	31.45	2.08	0.00	1.44	0.00	0.00
4/24/96	5:00	904.5	318	34.60%	0	8.65%	26.02	64.09	35.57	9.23	29.85	2.04	0.00	1.05	0.00	1.23

Run start data 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

Date	Time	Run time (h)	APR #						Liquor Analysis (Total Sugars)				
				Glycerol	Acetic	EtOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
4/3/96	21:00	416.5	260	0.00	3.81	0.00	0.00	0.00	124.25	64.31	14.28	41.60	4.19
4/4/96	5:00	424.5	261	0.00	3.23	0.00	0.00	0.00	127.04	63.87	15.76	42.00	6.10
4/4/96	21:00	440.5	263	0.00	3.12	0.00	0.00	0.00	118.01	62.67	13.67	41.21	0.00
4/5/96	5:00	448.5	264	0.00	2.98	0.00	0.00	0.00	120.95	67.69	14.49	43.99	0.00
4/5/96	21:00	464.5	266	0.00	3.73	0.00	0.00	0.00	117.98	70.27	16.14	45.64	5.77
4/6/96	5:00	472.5	267	0.00	4.18	0.00	0.00	0.00	126.16	67.36	15.52	44.06	5.35
4/6/96	21:00	488.5	269	0.00	3.00	0.00	0.00	0.00	122.23	67.94	15.37	45.20	5.15
4/7/96	5:00	496.5	270	0.00	2.07	0.00	0.00	0.00	137.24	71.78	17.10	47.46	0.00
4/7/96	21:00	512.5	272	0.00	4.46	0.00	0.00	0.00	117.41	65.63	14.33	41.16	11.83
4/8/96	5:00	520.5	273	0.00	4.40	0.00	0.00	0.00	124.06	68.10	14.60	42.22	0.00
4/8/96	21:00	536.5	275	0.00	4.18	0.00	0.00	0.00	128.45	65.74	14.26	40.85	4.72
4/9/96	5:00	544.5	276	0.00	3.55	0.00	0.00	0.00	127.82	70.35	15.02	41.81	2.69
4/9/96	21:00	560.5	278	0.00	3.73	0.00	0.00	0.00	124.83	67.15	14.04	41.22	10.65
4/10/96	5:00	568.5	279	0.00	3.18	0.00	0.00	0.00	128.35	67.18	14.12	41.45	5.12
4/11/96	5:00	592.5	282	0.00	3.57	0.00	0.00	0.00	120.01	64.73	13.43	39.62	10.36
4/12/96	5:00	616.5	285	0.00	2.97	0.00	0.00	0.00	124.35	64.47	14.25	40.53	12.62
4/13/96	5:00	640.5	286	0.00	1.88	0.00	0.00	0.00	103.51	45.93	10.07	29.23	0.00
4/13/96	21:00	656.5	288	0.00	1.97	0.00	0.00	0.00	119.23	56.16	12.40	37.90	0.00
4/14/96	5:00	664.5	289	0.00	2.16	0.00	0.00	0.00	127.00	61.62	13.56	40.33	12.94
4/14/96	21:00	680.5	291	0.00	2.56	0.00	0.00	0.00	120.31	60.29	14.13	39.33	11.53
4/15/96	5:00	688.5	292	0.00	2.23	0.00	0.00	0.00	134.44	61.46	14.66	40.54	0.00
4/15/96	21:00	704.5	293	0.00	2.38	0.00	0.00	0.00	124.00	62.76	14.19	40.75	12.47
4/16/96	5:00	712.5	294	0.00	2.49	0.00	0.00	0.00	126.36	62.88	14.35	40.08	12.41
4/16/96	13:20	720.8	295	0.00	2.96	0.00	0.00	0.00	132.37	54.59	5.64	35.46	0.00
4/17/96	5:00	736.5	296	0.00	1.79	0.00	0.00	0.00	113.99	50.37	10.69	36.33	0.00
4/17/96	21:00	752.5	299	0.00	2.91	0.00	0.00	0.00	122.70	60.82	13.20	39.43	0.00
4/18/96	5:30	761	300	0.00	2.19	0.00	0.00	0.00	142.78	58.95	15.58	42.05	0
4/18/96	21:00	776.5	302	0.00	3.10	0.00	0.00	0.00	140.42	58.51	11.43	35.81	0.00
4/19/96	5:00	784.5	303	0.00	2.95	0.00	0.00	0.00	124.90	61.30	11.57	37.63	0.00
4/19/96	21:00	800.5	305	0.00	3.70	0.00	0.00	0.00	125.40	64.61	13.59	39.81	0.00
4/20/96	5:00	808.5	306	0.00	1.81	0.00	0.00	0.00	129.96	63.71	13.83	40.23	0.00
4/20/96	21:00	824.5	308	0.00	3.40	0.00	0.00	0.00	127.92	63.75	14.45	44.36	0.00
4/21/96	5:30	833	309	0.00	2.86	0.00	0.00	0.00	134.86	70.63	16.00	48.39	0.00
4/21/96	21:00	848.5	311	0.00	3.77	0.00	0.00	0.00	133.74	64.43	13.50	40.69	0.00
4/22/96	5:00	856.5	312	0.00	3.77	0.00	0.00	0.00	135.42	68.07	14.07	42.09	0.00
4/22/96	21:00	872.5	314	0.00	0.29	0.00	0.00	0.00	119.92	60.86	13.09	40.03	0.00
4/23/96	5:00	880.5	315	0.00	3.91	0.00	0.00	0.00	115.16	61.24	13.05	39.38	0.00
4/23/96	21:00	896.5	317	0.00	3.97	0.00	0.00	0.00	115.79	61.81	13.33	40.53	0.00
4/24/96	5:00	904.5	318	0.00	3.05	0.00	0.00	0.00	126.59	65.71	15.32	43.19	5.77

Run start data 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

Date	Time	Run time (h)	APR #	Tot. Solids Oven (%)	TDS Liquid (%)	Ins. Solids (%)	Sample Wt. (g)	HPLC (g/L)								
								Glucose	Xylose	Gal.	Arab.	Man.	Cello.	Xylitol	Succinic	Lactic
4/25/96	5:00	928.5	320	33.95%	0	8.49%	27.52	31.59	20.85	6.05	26.54	9.20	0.00	0.99	0.00	1.94
4/25/96	21:00	944.5	322	31.59%	0	7.90%	25.42	37.06	24.35	6.82	27.85	0.00	0.00	0.97	0.00	0.00
4/26/96	5:00	952.5	323	36.33%	0	9.08%	26.61	56.26	29.62	8.44	30.38	0.00	0.00	1.55	0.00	2.26
4/26/96	13:00	960.5	324	33.36%	0	8.34%	25.42	36.19	24.99	6.97	29.97	0.00	0.00	1.32	0.00	2.32
4/27/96	5:00	976.5	325	34.91%	0	8.73%	25.83	37.13	23.31	6.25	28.47	0.00	0.00	1.89	0.00	0.00
4/29/96	5:00	1024.5	327	34.06%	0	8.51%	28.02	104.18	54.57	8.58	28.83	2.70	0.00	0.00	0.00	2.67
4/29/96	13:00	1032.5	329	34.14%	0	7.80%	27.89	86.65	49.63	0.79	27.06	0.00	0.00	2.99	0.00	2.64
4/29/96	21:00	1040.5	330	35.29%	0	8.82%	26.51	88.10	47.50	9.85	31.09	2.74	0.00	2.94	0.00	5.81

Run start data 17-Mar-96  
Run Name: CRADA Task 4  
Run ID#: P960314CF

Date	Time	Run time (h)	APR #						Liquor Analysis (Total Sugars)				
				Glycerol	Acetic	EtOH	HMF	furfural	Glucose	Xylose	Galactose	Arabinose	Mannose
4/25/96	5:00	928.5	320	0.00	2.01	0.00	0.00	0.00	146.61	62.02	12.96	41.93	2.75
4/25/96	21:00	944.5	322	0.00	2.37	0.00	0.00	0.00	109.81	53.13	10.95	35.61	2.79
4/26/96	5:00	952.5	323	0.00	3.45	0.00	0.00	0.00	146.84	59.32	12.24	37.97	0.00
4/26/96	13:00	960.5	324	0.00	3.76	0.00	0.00	0.00	114.93	55.98	10.73	36.16	1.47
4/27/96	5:00	976.5	325	0.00	5.92	0.00	0.00	0.00	138.24	55.43	9.68	34.85	0.00
4/29/96	5:00	1024.5	327	0.00	4.99	0.00	0.00	0.00	136.08	66.26	12.71	39.89	3.92
4/29/96	13:00	1032.5	329	0.00	4.22	0.00	0.00	0.00	118.98	60.78	2.77	35.92	0.00
4/29/96	21:00	1040.5	330	0.00	3.85	0.00	0.00	0.00	136.58	68.98	12.65	42.26	4.37

PDU Pretreatment Material Balance - PAD

Run #: P950314CF APR-214 Using P950314CF average feedstock composition  
 Date: 3/16/96  
 Time: 13:00

Run Conditions	Run Tank Temp (C)	68
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Input Data	SD	Row
Feed Flow Rate (kg/hr)	54.5	4.2
Steam In	2.3	1.7
Acid Flow Rate (kg/hr)	19.4	0.9
Water Flow Rate (kg/hr)	5.8	0.0
Water In	0.0	0.0
Water Out	17.0	1.8

SD	Row
0.9	
0.34	

Calculated Results	Pretreatment Solids Conc. (%)	38.50
	Solids Solubility (%)	78.47
Monomer: Total Sugar Ratio (%)		
	Glucose	67.75
	Mannose	65.50
	Galactose	70.25
	Arabinose	76.76
	Xylose	80.00

Carbon Balance: Pretreatment SD for raw feedstock used values generated for P950122CF

Component	Unpretreated				Pretreated												Std Dev		
	On Feed (% dry weight)	Carbon In (C-moles/hr)	SD	Std Err	In Solids		In Liquid		In Feed				Total						
					(% dry weight)	(C-moles/hr (% C in Feed))	(g/L/min)	(g/L/min)	(C-moles/hr)	(% C in Feed)	(g/L/min)	(C-moles/hr)	(% C in Feed)						
Composition				Composition				Composition				Composition							
Glucose	44.62	371.656	1.860	1112.303	62.2	111.557	30.0	1.0	232.677	74.8	122.4	242.856	65.3	16.5	1299.140	354.413	1531.820	95.4	13.6
Mannose	0	0.000			0	0.000	0.0		0.0	0	0	0.000	0.0			0.000	0.000		
Galactose	3.63	30.236	0.540	26.004	0.4	0.717	2.4	0.3	0.360	8.5	12.1	24.008	79.4	0.5	3.019	24.725	3.379	81.8	15.1
Xylose	18.39	153.177	2.230	493.181	2.5	4.484	2.9	0.7	1.858	41.2	62.9	124.801	81.5	9.9	445.882	131.501	447.830	85.8	18.6
Arabinose	12.13	101.055	1.170	159.436	1.6	2.870	2.8	0.2	0.256	31.7	41.3	81.944	81.1	3.6	76.906	84.814	77.162	83.9	13.6
Acetic Acid										4.1	8.135	0.1		0.255	2.5	1.4		0.2	0.0
Formic Acid										0	0.000	0.0		0.000					
Lactic Acid										0.4	0.794	0.1		0.002				0.794	0.002
Lignin	7.5	90.123	0.680	117.353	26.76	68.872	76.4	1.0	166.256	11.14	38.718	35.2	1.6	24.892				100.591	211.148
Furfural										0	0.000	0.0		0.004	2.5	2.2	0.9	0.2	0.1
HFHF										0	0.000	0.0		0.003					
Total	77.8	746.224	Std Dev =	43.664	82.7	188.500	25.3	Std Dev =	80.628	514.252	29.9	Std Dev =	43.013	3.6	0.5	Std Dev =	8.108	704.391	47.662

ignores protein

Std. Dev. = 6.46%

# PDU Pretreatment Material Balance - PAD

Run #: P06014CF APR-295 Using actual feedstock composition taken at same time as APR sample  
 Date: 4/16/96 SD from average feedstock data  
 Time: 13.00

Run Conditions	Run Tank Temp (°C)	98
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Input Data	SD	SD
Feed Flow Rate (kg/hr)	59.1	1.6
Moisture (%)	10.0	0.3
Acid Flow Rate (H <sub>2</sub> SO <sub>4</sub> ) (kg/hr)	14.5	0.1
Time to Run Tank (H <sub>2</sub> SO <sub>4</sub> ) (kg/hr)	6.2	0.0
Water & Solids (kg/hr)	0.0	0.0
Water & Solids (kg/hr)	16.6	1.8

Calculated Results	Pretreatment Solids Comp (%)	37.21
	Solids Substituted (%)	77.91
Monomer Total Sugar Ratio (%)	Glucose	41.77
	Fructose	66.90
	Galactose	71.10
	Mannose	78.88
	Starch	100.00

## Carbon Balance: Pretreatment SD for raw feedstock used values generated for P060422CF

Component	Unpretreated				Pretreated																
	On Feed (% dry weight)	Carbon (C-mole/hr)	In Soln				In Feed				In Feed				Total						
			In Soln		In Feed		In Feed		In Feed		In Feed		In Feed								
			(% dry weight)	(C-mole/hr)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)	(% C in Feed)						
SD	So Error	SD	So Error	SD	So Error	SD	So Error	SD	So Error	SD	So Error	SD	So Error	SD	So Error	Std Dev					
Glucose	45.78	393.056	1.850	447.939	51.22	97.129	24.7	1.0	379.100	81.8	132.37	280.299	71.3	16.5	1939.866		377.438	2318.967	96.0	13.3	
Mannose	0	0.000			0	0.000	0.0			0.0	0	0.000	0.0				0.000	0.000			
Galactose	3.18	27.303	0.540	22.426	0.36	0.683	2.5	6.3	0.410	4.0	5.64	11.943	43.7	0.5	2.213		12.626	2.624	46.2	19.0	
Ylucose	17.58	150.937	2.230	395.026	2.85	5.405	3.6	0.7	2.825	36.5	54.59	115.597	76.6	9.9	561.778		121.550	564.640	80.5	19.0	
Arabinose	10.87	93.327	1.170	111.785	1.98	3.755	4.0	0.2	0.677	25.5	35.46	75.089	80.5	3.6	109.717		78.843	110.394	84.5	14.8	
Acetic Acid										2.96	4.268	0.1		0.350	1.0	0.6		6.424	0.375		
Formic Acid										0	0.000	0.0		0.000							
Lactic Acid										0.87	1.842	0.1		0.031				1.842	0.031		
Lignin	7.5	91.789	0.680	80.732	25.21	68.609	74.7	1.0	392.553	18.73	35.648	38.0	0.3	12.516			104.253	405.069	113.6	24.6	
Furfural										0	0.000	0.0	0.0	0.004	0.6	0.5	0.2	0.2	0.0		
Water										0	0.000	0.0	0.0	0.004							
Total	76.6	756.411	Std Dev =	32.635	75.9	175.591	23.2	Std Dev =	27.849	526.680	69.6	Std Dev =	61.349	1.1	0.1	Std Dev =	0.736	703.373	84.328		

Ignored protein

Ignored protein SD = 8.69%

PDU Pre-treatment Material Balance - PAD

Run # P96014CF APR-329 Using a dry feedstock can produce  
Date 4/22/96  
Time 13:00

Run Conditions	Run Tank Temp (C)
	98

Input Data	SD	Calculated Results
Feed Flow Rate (kg/hr)	54.5	Pre-treatment Solids Conc. (%)
Steam (kg/hr)	10.5	Solids Solubility (%)
Acid Flow Rate (100-1 kg/hr)	12.0	Pre-treatment Solids Rate (kg/hr)
Water to Run Tank (100-1 kg/hr)	6.2	Glucose
Water to Run Tank (100-1 kg/hr)	0.0	Fructose
Water to Run Tank (100-1 kg/hr)	0.0	Galactose
Water to Run Tank (100-1 kg/hr)	15.6	Arabinose
		Mannose

Carbon Balance: Pre-treatment SD for raw feedstock used: values generated for P960122CF

Component	Unpretreated				Pretreated				Total				Std Dev			
	(% of Feed)	(% of Carbon)	(% of Carbon)	(% of Carbon)	(% of Feed)	(% of Carbon)	(% of Carbon)	(% of Carbon)	(% of Feed)	(% of Carbon)	(% of Carbon)	(% of Carbon)	(% of Feed)	(% of Carbon)	(% of Carbon)	(% of Carbon)
Glucose	44.62	371.656	1.660	417.053	61.9	110.197	29.7	1.0	432.072	86.6	119	250.416	47.4	16.5	1771.396	360.614
Mannose	0	0.000	0.000	0.000	0	0.000	0.0	0.0	0.000	0.0	0	0.000	0.0	0.0	0.000	0.000
Galactose	3.63	30.236	0.540	21.402	0.09	0.160	0.5	0.1	0.146	0.8	12.6	26.515	87.7	0.5	7.240	26.675
Xylose	18.39	153.177	2.230	375.082	0.86	1.531	1.0	0.7	1.548	49.6	60.6	127.944	83.5	9.9	581.712	129.995
Arabinose	12.13	101.035	1.170	108.055	0.73	1.300	1.3	0.2	0.162	27.1	42.6	89.645	88.7	3.6	129.899	90.944
Acetic Acid										4.22	8.880	0.1		0.712	1.0	0.5
Formic Acid										0	0.000	0.0		0.000		
Lactic Acid										2.64	5.555	0.1		0.278		
Ulgan	7.5	69.047	0.680	76.224	28.06	71.684	80.5	1.0	379.282	13.73	41.461	46.6	0.3	16.387		
Furfural										0	0.000	0.0	0.0	0.004	0.6	0.9
HMF										0	0.000	0.0	0.0	0.004	0.2	0.2
Total	77.7	745.151	Std Dev =	31.568	85.3	184.872	Std Dev =	28.929	550.417	73.9	Std Dev =	50.078	1.0	0.1	Std Dev =	0.117

ignores protein  
Std Dev = 8.60%



Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
16-Mar-96	2:00	-34.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	56.11	32.86	7.42	24.78	5.35	21.65
17-Mar-96	12:25	0		0.00%		0.00%	0.00	0.00%	0.00%	0.00	69.08	31.97	7.21	23.74	6.32	12.26
17-Mar-96	12:30	0														
17-Mar-96	18:00	5.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	68.90	32.85	7.44	24.45	6.22	14.12
18-Mar-96	2:00	13.5														
18-Mar-96	10:00	21.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.22	32.06	8.56	28.92	0.00	13.11
18-Mar-96	15:05	26.6														
18-Mar-96	18:00	29.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.19	32.06	8.94	29.49	0.00	12.48
19-Mar-96	2:00	37.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.61	31.06	8.74	28.53	0.00	12.05
19-Mar-96	10:00	45.5														
19-Mar-96	18:00	53.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.71	31.11	8.12	27.41	0.00	11.34
20-Mar-96	2:20	61.8		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.84	30.54	7.92	26.45	0.00	11.05
20-Mar-96	7:45	67.2														
20-Mar-96	10:00	69.5														
20-Mar-96	18:00	77.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.58	30.51	8.03	26.97	0.00	10.79
21-Mar-96	2:00	85.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.54	29.95	7.73	26.00	0.00	10.64
21-Mar-96	10:00	93.5														
21-Mar-96	18:00	101.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.89	31.02	8.19	24.81	1.76	10.94
22-Mar-96	2:00	109.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.73	30.59	8.07	26.50	0.00	11.25
22-Mar-96	10:00	117.5														
22-Mar-96	18:00	125.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.25	26.66	7.24	24.06	0.00	11.64
23-Mar-96	2:00	133.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.21	27.38	7.47	24.71	0.00	12.25
23-Mar-96	10:00	141.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.06	25.99	7.23	23.91	0.00	10.04
23-Mar-96	18:00	149.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.00	26.39	7.38	24.45	0.00	10.17
24-Mar-96	2:00	157.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.80	24.22	6.86	22.48	0.00	9.33
24-Mar-96	10:00	165.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.65	23.48	6.82	21.14	1.09	8.10
24-Mar-96	18:00	173.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.59	23.76	6.67	21.66	0.98	9.05
25-Mar-96	2:00	181.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.79	22.99	6.03	21.08	1.09	9.05
25-Mar-96	10:00	189.5														
25-Mar-96	18:00	197.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.65	22.36	5.42	22.80	0.00	8.21
26-Mar-96	2:00	205.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	6.17	24.87	6.00	24.21	0.00	8.78
26-Mar-96	10:00	213.5														
26-Mar-96	18:00	221.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.37	27.63	6.38	25.48	0.00	10.18
27-Mar-96	2:00	229.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	8.96	28.86	7.06	26.46	0.00	10.47
27-Mar-96	10:00	237.5														
27-Mar-96	13:20	240.8														
27-Mar-96	18:00	245.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.97	27.76	6.85	24.81	0.00	10.34
27-Mar-96	22:00	249.5														
28-Mar-96	2:00	253.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.88	27.97	6.89	24.17	0.00	10.47

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
16-Mar-96	2:00	34.5	1.11	0.00	2.28	0.96	3.42	8.87	0.12	0.00
17-Mar-96	12:25	0	1.24	0.00	2.46	0.44	3.55	0.67	0.21	0.03
17-Mar-96	12:30	0								
17-Mar-96	18:00	5.5	1.23	0.00	2.45	0.58	3.62	2.03	0.19	0.00
18-Mar-96	2:00	13.5								
18-Mar-96	10:00	21.5	1.68	0.00	3.18	4.24	4.13	36.99	0.00	0.00
18-Mar-96	15:05	26.6								
18-Mar-96	18:00	29.5	1.63	0.00	3.01	4.27	4.05	39.61	0.00	0.00
19-Mar-96	2:00	37.5	1.60	0.00	2.85	4.00	3.79	38.94	0.00	0.00
19-Mar-96	10:00	45.5								
19-Mar-96	18:00	53.5	0.00	0.00	2.04	3.16	3.16		0.00	0.00
20-Mar-96	2:20	61.8	0.00	0.00	1.91	3.02	2.81		0.00	0.00
20-Mar-96	7:45	67.2								
20-Mar-96	10:00	69.5								
20-Mar-96	18:00	77.5	1.19	0.00	1.92	3.04	3.03	36.02	0.00	0.00
21-Mar-96	2:00	85.5	1.75	0.00	2.66	4.47	3.94	36.94	0.00	0.00
21-Mar-96	10:00	93.5								
21-Mar-96	18:00	101.5	0.00	0.00	2.34	4.21	4.16		0.00	0.00
22-Mar-96	2:00	109.5	0.00	0.00	2.12	3.97	3.86		0.00	0.00
22-Mar-96	10:00	117.5								
22-Mar-96	18:00	125.5	1.28	0.00	2.32	5.09	3.50	35.81	0.00	0.00
23-Mar-96	2:00	133.5	1.08	0.00	2.06	3.67	3.17	35.92	0.00	0.00
23-Mar-96	10:00	141.5	1.49	0.00	2.59	4.72	3.83	36.16	0.00	0.00
23-Mar-96	18:00	149.5	1.62	0.00	2.84	5.04	4.12	37.08	0.00	0.00
24-Mar-96	2:00	157.5	1.42	0.00	2.40	4.24	3.68	34.07	0.00	0.00
24-Mar-96	10:00	165.5	1.56	0.00	3.15	5.59	4.99	34.40	0.00	0.00
24-Mar-96	18:00	173.5	1.49	0.00	2.93	5.45	5.10	35.23	0.00	0.00
25-Mar-96	2:00	181.5	1.57	0.00	2.96	5.79	5.42	35.29	0.00	0.00
25-Mar-96	10:00	189.5								
25-Mar-96	18:00	197.5	1.06	0.00	1.55	3.07	3.88	35.23	0.00	0.00
26-Mar-96	2:00	205.5	1.48	0.00	2.32	4.08	4.58	36.68	0.00	0.00
26-Mar-96	10:00	213.5								
26-Mar-96	18:00	221.5	1.28	0.00	2.45	3.71	3.94	38.29	0.00	0.00
27-Mar-96	2:00	229.5	1.54	0.00	3.08	4.02	4.53	38.74	0.00	0.00
27-Mar-96	10:00	237.5								
27-Mar-96	13:20	240.8								
27-Mar-96	18:00	245.5	1.71	0.00	3.55	4.90	4.52	38.15	0.00	0.00
27-Mar-96	22:00	249.5								
28-Mar-96	2:00	253.5	1.46	0.00	3.16	4.36	4.02	38.89	0.00	0.00

Run ID#: P960314CF

**Vessel: V-455A**

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass CFUs	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	DCW (g/L)	% Viability	Enz. Actv. FPU/mL	Enz. Digest	Contamination			pH	
												Scope	Liquid	Plate	Floor	Micro
28-Mar-96	6:00	257.5			3.48	33.2	1.61									5.11
28-Mar-96	9:00	260.5		4.00E+07	2.65	34.6	1.67						rods			5.07
28-Mar-96	18:00	269.5			1	32.3	1.62									5.11
28-Mar-96	22:00	273.5			2.97	33.6	1.55									5.10
29-Mar-96	2:00	277.5			2.91	36.5	1.52									5.11
29-Mar-96	9:00	284.5		4.47E+07									rods			
29-Mar-96	10:00	285.5			2.89	34.45	1.43									5.03
29-Mar-96	18:00	293.5			2.75	31.8	1.33									5.11
30-Mar-96	2:00	301.5			2.96	31.5	1.29									4.99
30-Mar-96	10:00	309.5			0.38	34.75	1.26									5.07
30-Mar-96	18:00	317.5			3.22	31.7	1.22									5.03
31-Mar-96	2:00	325.5			3.28	27	1.15									5.02
31-Mar-96	10:00	333.5			2.68	30.7	1.1									5.05
31-Mar-96	18:00	341.5			2.74	30.85	1.07									5.04
1-Apr-96	2:00	349.5			2.93	30.05	1.01									4.97
1-Apr-96	10:00	357.5		4.35E+07	2.64	31.1	1.04									5.10
1-Apr-96	18:00	365.5			2.28	30.1	1.03									5.15
2-Apr-96	2:00	373.5			1.99	30	1.03									5.06
2-Apr-96	10:00	381.5		5.20E+07	1.92	30.5	0.99									4.95
2-Apr-96	18:00	389.5			2.19	31.05	1									5.03
3-Apr-96	2:00	397.5			2.27	30	1.07									5.10
3-Apr-96	10:00	405.5		5.85E+07	2.14	30.2	1									5.06
3-Apr-96	17:30	413.0			1.95	33.65	1.15									4.81
4-Apr-96	2:00	421.5			1.99	30.9	1.28									4.82
4-Apr-96	10:00	429.5			2.2	32.7	1.51									4.83
4-Apr-96	18:00	437.5			2.34	31.05	1.82									4.95
5-Apr-96	2:30	446.0			2.13	31.65	2.33									4.88
5-Apr-96	10:00	453.5		4.65E+07	2.7	31.95	2.92									4.86
5-Apr-96	18:00	461.5			3.65	31.55	3.38									5.06
6-Apr-96	2:00	469.5			5.98	27.95	3.82									4.85
6-Apr-96	10:00	477.5		3.85E+07	5.64	28.55	4.49									4.90
6-Apr-96	18:00	485.5			5.36	29.45	4.6									4.97
7-Apr-96	2:00	493.5			5.21	27.35	4.58									4.91
7-Apr-96	10:00	501.5			4.76	31.75	4.68									4.92
7-Apr-96	18:00	509.5			3.98	30.9	3.92									4.94
8-Apr-96	2:00	517.5			4.06	23.3	3.52									4.94
8-Apr-96	10:00	525.5		4.70E+07	3.36	30.2	3.14									4.92
8-Apr-96	18:00	533.5			3.16	30.7	2.76									4.93
9-Apr-96	2:00	541.5			2.29	30.55	2.35									4.94

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
28-Mar-96	6:00	257.5														
28-Mar-96	9:00	260.5														
28-Mar-96	18:00	269.5														
28-Mar-96	22:00	273.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.82	27.95	6.92	23.37	0.00	9.87
29-Mar-96	2:00	277.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.08	29.41	7.20	24.35	0.00	9.87
29-Mar-96	9:00	284.5														
29-Mar-96	10:00	285.5														
29-Mar-96	18:00	293.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.81	31.43	7.58	25.80	0.00	10.35
30-Mar-96	2:00	301.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.29	31.84	7.82	26.67	0.00	10.24
30-Mar-96	10:00	309.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.16	30.15	7.54	25.37	0.00	10.03
30-Mar-96	18:00	317.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.84	28.89	7.43	24.69	0.00	9.71
31-Mar-96	2:00	325.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.92	28.20	7.77	24.71	1.39	10.13
31-Mar-96	10:00	333.5														
31-Mar-96	18:00	341.5														
1-Apr-96	2:00	349.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.85	29.10	7.91	26.66	0.00	10.20
1-Apr-96	10:00	357.5														
1-Apr-96	18:00	365.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.73	28.22	7.82	26.02	0.00	9.95
2-Apr-96	2:00	373.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.65	28.87	8.03	26.57	0.00	10.23
2-Apr-96	10:00	381.5														
2-Apr-96	18:00	389.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.80	28.14	7.92	24.68	1.47	10.95
3-Apr-96	2:00	397.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.28	28.69	8.08	24.93	1.50	10.52
3-Apr-96	10:00	405.5														
3-Apr-96	17:30	413.0		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.08	28.53	8.04	24.47	0.00	10.57
4-Apr-96	2:00	421.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.69	27.20	7.69	23.08	0.00	10.27
4-Apr-96	10:00	429.5														
4-Apr-96	18:00	437.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.57	26.93	7.49	22.36	0.00	10.77
5-Apr-96	2:30	446.0		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.51	26.61	7.50	21.48	0.00	10.92
5-Apr-96	10:00	453.5														
5-Apr-96	18:00	461.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	6.19	25.95	7.45	19.47	0.00	10.69
6-Apr-96	2:00	469.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	8.12	26.50	7.48	18.95	0.00	11.04
6-Apr-96	10:00	477.5														
6-Apr-96	18:00	485.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	7.29	27.30	7.59	17.68	0.00	10.96
7-Apr-96	2:00	493.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	7.34	27.67	7.75	18.28	0.00	11.24
7-Apr-96	10:00	501.5														
7-Apr-96	18:00	509.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	6.63	27.89	7.78	19.52	0.00	11.27
8-Apr-96	2:00	517.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	6.23	27.10	7.36	18.84	0.00	10.41
8-Apr-96	10:00	525.5														
8-Apr-96	18:00	533.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	5.55	27.48	7.56	20.96	0.00	10.71
9-Apr-96	2:00	541.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.96					10.97

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	lurfural
28-Mar-96	6:00	257.5								
28-Mar-96	9:00	260.5								
28-Mar-96	18:00	269.5	1.12	0.00	2.76	3.73	3.42	39.15	0.00	0.00
28-Mar-96	22:00	273.5								
29-Mar-96	2:00	277.5	1.47	0.00	3.09	4.37	4.01	38.03	0.00	0.00
29-Mar-96	9:00	284.5								
29-Mar-96	10:00	285.5								
29-Mar-96	18:00	293.5	1.56	0.00	2.93	4.61	4.06	33.48	0.00	0.00
30-Mar-96	2:00	301.5	1.29	0.00	2.54	4.20	3.76	35.48	0.00	0.00
30-Mar-96	10:00	309.5	1.22	0.00	2.44	4.20	3.63	35.59	0.00	0.00
30-Mar-96	18:00	317.5	1.25	0.00	2.41	4.14	3.71	34.94	0.00	0.00
31-Mar-96	2:00	325.5	0.76	0.00	1.56	3.18	2.98	34.23	0.00	0.00
31-Mar-96	10:00	333.5								
31-Mar-96	18:00	341.5								
1-Apr-96	2:00	349.5	1.04	0.00	1.90	3.90	3.52	34.37	0.00	0.00
1-Apr-96	10:00	357.5								
1-Apr-96	18:00	365.5	0.77	0.00	1.52	3.25	3.09	33.39	0.00	0.00
2-Apr-96	2:00	373.5	0.97	0.00	1.82	3.73	3.41	33.03	0.00	0.00
2-Apr-96	10:00	381.5								
2-Apr-96	18:00	389.5	1.15	0.00	2.08	4.15	3.63	32.75	0.00	0.00
3-Apr-96	2:00	397.5	0.92	0.00	1.77	3.64	3.25	32.22	0.00	0.00
3-Apr-96	10:00	405.5								
3-Apr-96	17:30	413.0	1.03	0.00	2.23	3.82	3.46	32.98	0.00	0.00
4-Apr-96	2:00	421.5	1.37	0.00	3.08	4.69	4.17	33.04	0.00	0.00
4-Apr-96	10:00	429.5								
4-Apr-96	18:00	437.5	1.09	0.00	3.69	4.04	4.17	33.65	0.00	0.00
5-Apr-96	2:30	446.0	1.01	0.00	4.61	3.86	4.48	33.84	0.00	0.00
5-Apr-96	10:00	453.5								
5-Apr-96	18:00	461.5	1.43	0.00	7.52	4.80	6.39	31.87	0.00	0.00
6-Apr-96	2:00	469.5	1.49	0.00	8.79	5.15	6.95	30.45	0.00	0.00
6-Apr-96	10:00	477.5								
6-Apr-96	18:00	485.5	1.57	0.00	10.67	5.88	8.29	27.89	0.00	0.00
7-Apr-96	2:00	493.5	1.50	0.00	10.22	5.71	8.08	29.04	0.00	0.00
7-Apr-96	10:00	501.5								
7-Apr-96	18:00	509.5	1.36	0.00	8.42	4.96	6.99	31.47	0.00	0.00
8-Apr-96	2:00	517.5	1.53	0.00	7.95	4.96	6.67	31.93	0.00	0.00
8-Apr-96	10:00	525.5								
8-Apr-96	18:00	533.5	1.54	0.00	6.12	4.54	5.65	33.57	0.00	0.00
9-Apr-96	2:00	541.5								

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EIOH	HMF	furfural
9-Apr-96	10:00	549.5								
9-Apr-96	18:00	557.5	1.67	0.00	4.24	4.25	4.80	33.65	0.00	0.00
10-Apr-96	2:15	565.8	1.78	0.00	3.91	4.34	4.57	33.05	0.00	0.00
10-Apr-96	10:00	573.5								
10-Apr-96	18:20	581.8								
11-Apr-96	2:10	589.7								
11-Apr-96	10:15	597.8								
11-Apr-96	18:15	605.7	1.65	0.00	2.62	3.57	3.61	33.11	0.00	0.00
12-Apr-96	2:10	613.7	1.67	0.00	2.53	3.45	3.31	32.19	0.00	0.00
12-Apr-96	10:00	621.5								
12-Apr-96	18:20	629.8	1.58	0.00	2.49	3.35	3.26	32.95	0.00	0.00
13-Apr-96	2:30	638.0	1.62	0.00	2.99	3.44	3.46	33.48	0.00	0.00
13-Apr-96	10:00	645.5								
13-Apr-96	18:00	653.5	1.47	0.00	4.00	3.43	3.97	30.29	0.00	0.00
14-Apr-96	2:00	661.5	1.56	0.00	4.24	3.79	4.17	31.90	0.00	0.00
14-Apr-96	10:00	669.5								
14-Apr-96	18:00	677.5	1.14	0.00	3.44	4.29	3.80	31.32	0.00	0.00
15-Apr-96	2:00	685.5	1.21	0.00	3.42	4.80	3.86	30.33	0.00	0.00
15-Apr-96	10:00	693.5								
15-Apr-96	18:00	701.5	0.94	0.00	2.62	5.40	3.35	32.27	0.00	0.00
16-Apr-96	2:00	709.5	1.11	0.00	2.66	5.81	3.54	33.09	0.00	0.00
16-Apr-96	10:00	717.5								
16-Apr-96	18:00	725.5	1.16	0.00	2.39	5.86	3.49	33.61	0.00	0.00
17-Apr-96	2:00	733.5	1.17	0.00	2.20	5.48	3.31	32.85	0.00	0.00
17-Apr-96	10:00	741.5								
17-Apr-96	18:00	749.5	1.12	0.00	2.06	4.71	3.00	34.57	0.00	0.00
18-Apr-96	3:00	758.5	1.16	0.00	1.96	4.18	2.80	35.32	0.00	0.00
18-Apr-96	10:00	765.5								
18-Apr-96	10:30	766.0								
18-Apr-96	18:00	773.5	1.27	0.00	2.17	3.82	2.86	36.94	0.00	0.00
19-Apr-96	2:00	781.5	1.29	0.00	2.12	3.46	2.76	37.14	0.00	0.00
19-Apr-96	10:00	789.5								
19-Apr-96	18:00	797.5	1.38	0.00	2.29	3.01	2.95	37.87	0.00	0.00
20-Apr-96	2:00	805.5	1.53	0.00	4.28	4.63	5.30	35.53	0.00	0.00
20-Apr-96	10:15	813.8								
20-Apr-96	18:00	821.5	1.53	0.00	2.38	2.87	3.20	41.15	0.00	0.00
21-Apr-96	2:15	829.7	1.49	0.00	2.29	2.70	3.11	42.92	0.00	0.00
21-Apr-96	10:00	837.5								
21-Apr-96	18:00	845.5	1.42	0.00	2.30	2.57	3.02	45.12	0.00	0.00

Run ID#: P960314CF

[illegible]

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
22-Apr-96	2:00	853.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.55	24.01	7.10	22.28	0.00	12.38
22-Apr-96	10:00	861.5														
22-Apr-96	18:00	869.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.07	24.81	7.27	22.76	0.00	12.24
23-Apr-96	2:00	877.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.87	24.96	7.36	22.85	0.00	12.52
23-Apr-96	10:00	885.5														
23-Apr-96	10:30	886.0														
23-Apr-96	18:00	893.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.19	24.00	7.21	22.76	0.00	11.89
24-Apr-96	2:00	901.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.66	23.12	6.97	22.25	0.00	11.83
24-Apr-96	10:00	909.5														
24-Apr-96	18:00	917.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.13	23.20	6.93	22.35	0.00	11.78
25-Apr-96	2:00	925.5														
25-Apr-96	10:00	933.5														
25-Apr-96	18:00	941.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.27	21.09	6.69	22.48	0.00	11.42
26-Apr-96	2:00	949.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.95	20.82	6.67	22.61	0.00	11.56
26-Apr-96	10:00	957.5														
26-Apr-96	18:00	965.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.36	19.44	6.36	21.94	0.00	11.01
27-Apr-96	2:00	973.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	18.51	6.16	20.78	0.00	11.21
27-Apr-96	10:15	981.8														
27-Apr-96	18:00	989.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.95	16.19	5.64	17.47	0.00	10.17
28-Apr-96	2:00	997.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.17	15.53	5.58	0.65	0.00	10.28
28-Apr-96	10:00	1005.5														
28-Apr-96	18:00	1013.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.24	14.03	5.10	8.11	0.00	9.57
29-Apr-96	2:00	1021.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	4.23	14.93	5.16	6.15	0.00	9.69
29-Apr-96	9:40	1029.2		0.00%		0.00%	0.00	0.00%	0.00%	0.00	19.05	20.60	5.75	7.86	0.89	10.30
29-Apr-96	18:00	1037.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	29.31	24.24	6.01	9.81	1.25	10.60

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
22-Apr-96	2:00	853.5	1.54	0.00	2.36	2.65	3.13	47.05	0.00	0.00
22-Apr-96	10:00	861.5								
22-Apr-96	18:00	869.5	1.56	0.00	2.26	2.62	3.14	47.95	0.00	0.00
23-Apr-96	2:00	877.5	1.43	0.00	2.10	2.41	3.06	48.29	0.00	0.00
23-Apr-96	10:00	885.5								
23-Apr-96	10:30	886.0								
23-Apr-96	18:00	893.5	1.45	0.00	2.28	2.58	3.35	49.01	0.00	0.00
24-Apr-96	2:00	901.5	1.35	0.00	2.13	2.47	3.14	48.74	0.00	0.00
24-Apr-96	10:00	909.5								
24-Apr-96	18:00	917.5	1.43	0.00	2.27	2.57	3.33	48.79	0.00	0.00
25-Apr-96	2:00	925.5								
25-Apr-96	10:00	933.5								
25-Apr-96	18:00	941.5	1.39	0.00	2.40	2.74	3.34	48.97	0.00	0.00
26-Apr-96	2:00	949.5	1.29	0.00	2.25	2.63	3.25	48.89	0.00	0.00
26-Apr-96	10:00	957.5								
26-Apr-96	18:00	965.5	1.22	0.00	2.38	2.52	3.26	42.76	0.00	0.00
27-Apr-96	2:00	973.5	1.17	0.00	2.67	2.49	3.43	45.98	0.00	0.00
27-Apr-96	10:15	981.8								
27-Apr-96	18:00	989.5	1.09	0.55	4.38	2.85	4.47	45.50	0.00	0.00
28-Apr-96	2:00	997.5	1.17	0.70	6.28	2.92	5.76	45.80	0.00	0.00
28-Apr-96	10:00	1005.5								
28-Apr-96	18:00	1013.5	2.58	0.86	10.89	3.02	9.24	45.85	0.00	0.00
29-Apr-96	2:00	1021.5	2.71	0.92	12.63	3.26	10.65	43.47	0.00	0.00
29-Apr-96	9:40	1029.2	1.68	0.00	13.11	3.25	11.02	38.49	0.00	0.00
29-Apr-96	18:00	1037.5	1.74	0.00	10.80	2.80	9.07	27.59	0.00	0.00

Run ID#: P960314CF

Vessel: V-455B

Date	Time	Run time (h)	O.D 600 nm	Cell Mass CFUs	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	DCW (g/L)	% Viability	Enz. Activ. FPU/mL	Enz. Digest	Contamination			pH	
												Scope	Liquid	Plate	Floor	Micro
18-Mar-96	10:00	21.5	1.26E+08										clean			
3/18/96	14:00	25.5			0.711	32.25	1.34								4.94	
3/18/96	18:00	29.5			0.629	45.25	1.34								5.06	
18-Mar-96	18:05	29.583333														
3/19/96	2:00	37.5			0.576	42.9	1.32								5.04	
3/19/96	10:00	45.5	1.17E+08		0.54	40.15	1.33					clean			5.02	
3/19/96	18:00	53.5			0.56	44.2	1.34								5.09	
3/20/96	2:20	61.833333			0.53	41.6	1.3								5.12	
3/20/96	7:45	67.25	7.95E+07									clean				
3/20/96	10:00	69.5			0.5	41.7	1.25								5.13	
3/20/96	18:00	77.5			0.5	44.3	1.24								5.04	
3/21/96	2:00	85.5			0.491	45.15	1.21								5.06	
3/21/96	10:00	93.5	6.95E+07		0.4	43.2	1.16					clean			5.04	
3/21/96	18:00	101.5			0.14	38.95	1.16								5.02	
3/22/96	2:00	109.5			0.475	39.8	1.11								5.01	
3/22/96	10:00	117.5	6.50E+07		0.48	36.6	1.08					clean			5.11	
3/22/96	18:00	125.5			0.33	35.5	1.05								4.93	
3/23/96	2:00	133.5			0.38	39.9	1.08								5.09	
3/23/96	10:00	141.5			0.41	36.7	1.08								4.99	
3/23/96	18:00	149.5			0.33	42	1.25								5.02	
3/24/96	2:00	157.5			0.33		1.05								5.08	
3/24/96	10:00	165.5			0.33	36.4	1.06								5.05	
3/24/96	18:00	173.5			0.36	38.6	1.11								5.10	
3/25/96	2:00	181.5			0.39	41.5	1.04								5.04	
3/25/96	10:00	189.5	8.30E+07		0.337	38.9	1.05					rods			5.05	
3/25/96	18:00	197.5			0.43	40.6	1.05								5.11	
3/26/96	2:00	205.5			0.34	38.5	1.03								5.05	
3/26/96	10:00	213.5	6.15E+07		0.69	38.8	1.12					rods			5.14	
3/26/96	18:00	221.5			0.27	40	1.26								5.05	
3/27/96	2:00	229.5			0.29	37.7	1.17								5.15	
3/27/96	10:00	237.5	6.75E+07		0.766	38.08	1.32					rods			5.08	
3/27/96	18:00	245.5			0.489	37.35	1.35								5.09	
3/28/96	2:00	253.5			0.435	38.6	1.37								5.12	
3/28/96	9:00	260.5	6.05E+07		0.46	39.05	1.42					rods			5.09	
3/28/96	18:00	269.5			0.35	38.3	1.45								5.07	
3/29/96	2:00	277.5			0.32	40.9	1.52								5.16	
3/29/96	9:00	284.5	6.80E+07									rods				
3/29/96	10:00	285.5			0.46	38.3	1.47								5.10	
3/29/96	18:00	293.5			0.37	36.55	1.42								5.15	

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
18-Mar-96	10:00	21.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.58	30.49	8.22	27.58	0.00	11.95
3/18/96	14:00	25.5														
3/18/96	18:00	29.5														
18-Mar-96	18:05	29.583333		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.56	28.04	8.07	26.53	0.00	11.12
3/19/96	2:00	37.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.21	26.69	8.19	26.84	0.00	11.31
3/19/96	10:00	45.5														
3/19/96	18:00	53.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.72	25.05	7.91	25.92	0.00	10.01
3/20/96	2:20	61.833333		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.47	24.52	7.77	25.40	0.00	9.71
3/20/96	7:45	67.25														
3/20/96	10:00	69.5														
3/20/96	18:00	77.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.87	25.23	7.96	25.77	0.00	9.93
3/21/96	2:00	85.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.37	25.27	7.80	25.28	0.00	9.68
3/21/96	10:00	93.5														
3/21/96	18:00	101.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.66	25.42	7.73	24.31	0.00	9.45
3/22/96	2:00	109.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.37	26.90	8.12	25.59	0.00	10.03
3/22/96	10:00	117.5														
3/22/96	18:00	125.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.17	25.83	7.61	24.51	0.00	12.03
3/23/96	2:00	133.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.08	25.89	7.77	24.74	0.00	11.21
3/23/96	10:00	141.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.05	25.20	7.69	24.43	0.00	9.26
3/23/96	18:00	149.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.00	25.00	7.81	24.61	0.00	9.17
3/24/96	2:00	157.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.95	24.62	7.71	24.26	0.00	9.06
3/24/96	10:00	165.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.79	23.57	7.58	23.63	0.00	8.06
3/24/96	18:00	173.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.92	23.94	7.75	24.06	0.00	8.74
3/25/96	2:00	181.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.78	23.38	7.74	23.90	0.00	8.61
3/25/96	10:00	189.5														
3/25/96	18:00	197.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.86	22.31	7.63	23.72	0.00	0.00
3/26/96	2:00	205.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.57	21.56	7.23	22.94	0.00	0.00
3/26/96	10:00	213.5														
3/26/96	18:00	221.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.04	23.53	7.31	24.18	0.00	8.73
3/27/96	2:00	229.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.76	23.55	7.31	24.28	0.00	8.29
3/27/96	10:00	237.5														
3/27/96	18:00	245.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.23	24.63	7.09	24.30	0.00	8.69
3/28/96	2:00	253.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.91	25.05	7.03	24.01	0.00	8.91
3/28/96	9:00	260.5														
3/28/96	18:00	269.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.99	24.71	6.81	22.95	0.00	8.58
3/29/96	2:00	277.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.27	25.86	7.04	23.63	0.00	9.02
3/29/96	9:00	284.5														
3/29/96	10:00	285.5														
3/29/96	18:00	293.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.21	26.78	7.12	23.89	0.00	9.17

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
18-Mar-96	10:00	21.5	1.46	0.00	2.76	3.74	3.75	36.67	0.00	0.00
3/18/96	14:00	25.5								
3/18/96	18:00	29.5								
18-Mar-96	18:05	29.583333	1.58	0.00	2.87	4.34	3.94	38.62	0.00	0.00
3/19/96	2:00	37.5	1.78	0.00	3.07	4.94	4.16	40.86	0.00	0.00
3/19/96	10:00	45.5								
3/19/96	18:00	53.5	0.00	0.00	2.15	3.73	3.01		0.00	0.00
3/20/96	2:20	61.833333	0.00	0.00	2.04	3.60	2.96		0.00	0.00
3/20/96	7:45	67.25								
3/20/96	10:00	69.5								
3/20/96	18:00	77.5	2.38	0.00	2.78	5.07	4.11	41.98	0.00	0.00
3/21/96	2:00	85.5	1.68	0.00	2.36	4.45	3.74	42.60	0.00	0.00
3/21/96	10:00	93.5								
3/21/96	18:00	101.5	0.00	0.00	2.43	4.62	4.44		0.00	0.00
3/22/96	2:00	109.5	0.00	0.00	2.26	4.41	3.98		0.00	0.00
3/22/96	10:00	117.5								
3/22/96	18:00	125.5	1.81	0.00	2.64	5.13	4.13	40.08	0.00	0.00
3/23/96	2:00	133.5	1.21	0.00	1.69	3.64	3.12	39.01	0.00	0.00
3/23/96	10:00	141.5	1.72	0.00	2.38	4.96	3.96	40.05	0.00	0.00
3/23/96	18:00	149.5	1.82	0.00	2.43	5.18	4.09	40.58	0.00	0.00
3/24/96	2:00	157.5	1.78	0.00	2.47	5.20	4.12	39.80	0.00	0.00
3/24/96	10:00	165.5	1.74	0.00	3.02	6.08	5.02	39.54	0.00	0.00
3/24/96	18:00	173.5	1.78	0.00	3.04	6.17	5.05	41.58	0.00	0.00
3/25/96	2:00	181.5	1.85	0.00	0.06	6.28	5.14	42.47	0.00	0.00
3/25/96	10:00	189.5								
3/25/96	18:00	197.5	1.59	0.00	2.00	4.61	3.71	41.66	0.00	0.00
3/26/96	2:00	205.5	1.45	0.00	1.80	4.19	3.51	40.40	0.00	0.00
3/26/96	10:00	213.5								
3/26/96	18:00	221.5	1.49	0.00	2.05	4.24	3.95	42.17	0.00	0.00
3/27/96	2:00	229.5	1.48	0.00	1.98	4.17	3.97	42.88	0.00	0.00
3/27/96	10:00	237.5								
3/27/96	18:00	245.5	1.33	0.00	2.19	3.73	4.00	41.74	0.00	0.00
3/28/96	2:00	253.5	1.59	0.00	2.63	4.26	4.35	41.63	0.00	0.00
3/28/96	9:00	260.5								
3/28/96	18:00	269.5	1.76	0.00	3.23	5.03	4.79	40.66	0.00	0.00
3/29/96	2:00	277.5	1.49	0.00	2.82	4.29	4.31	41.99	0.00	0.00
3/29/96	9:00	284.5								
3/29/96	10:00	285.5								
3/29/96	18:00	293.5	1.42	0.00	2.78	4.30	4.13	39.80	0.00	0.00

Run ID#: P960314CF

**Vessel: V-455B**

[illegible]

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
3/30/96	2:00	301.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.40	29.34	7.84	25.33	0.00	9.94
3/30/96	10:00	309.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.99	28.17	7.40	24.53	0.00	9.57
3/30/96	18:00	317.5														
3/31/96	2:00	325.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.13	27.18	7.49	24.53	0.00	9.35
3/31/96	10:00	333.5														
31-Mar-96	18:00	341.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.25	27.14	7.21	23.46	0.00	8.91
1-Apr-96	2:00	349.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.04	27.37	7.55	24.82	0.00	9.52
1-Apr-96	10:00	357.5														
1-Apr-96	18:00	365.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.28	26.50	7.61	24.68	0.00	9.62
2-Apr-96	2:00	373.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.33	27.36	7.92	25.47	0.00	9.90
2-Apr-96	10:00	381.5														
2-Apr-96	18:00	389.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.20	27.11	7.88	25.61	0.00	10.25
3-Apr-96	2:00	397.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.06	27.08	7.95	25.39	0.00	10.35
3-Apr-96	10:00	405.5														
3-Apr-96	17:30	413		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.94	26.56	7.77	22.36	0.00	9.99
4-Apr-96	2:00	421.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.92	26.55	7.83	21.63	0.00	10.23
4-Apr-96	10:00	429.5														
4-Apr-96	18:00	437.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.96	24.49	7.25	17.55	0.00	0.00
5-Apr-96	2:30	446		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.02	24.55	7.22	16.29	0.00	10.61
5-Apr-96	10:00	453.5														
5-Apr-96	18:00	461.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.11	23.83	6.95	13.31	0.00	10.55
6-Apr-96	2:00	469.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.00	23.69	6.79	12.24	0.00	10.54
6-Apr-96	10:00	477.5														
6-Apr-96	18:00	485.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.32	24.09	6.94	11.12	0.00	10.35
7-Apr-96	2:00	493.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.44	24.57	7.02	10.50	0.00	10.78
7-Apr-96	10:00	501.5														
7-Apr-96	18:00	509.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.25	24.96	7.06	11.11	0.00	10.34
8-Apr-96	2:00	517.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.15	24.63	6.89	11.46	0.00	10.36
8-Apr-96	10:00	525.5														
8-Apr-96	18:00	533.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.17	24.78	7.01	13.37	0.00	10.05
9-Apr-96	2:00	541.5														
9-Apr-96	10:00	549.5														
9-Apr-96	18:00	557.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.03	26.03	6.98	16.40	0.00	10.65
10-Apr-96	2:15	565.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.73	27.34	7.34	17.90	0.00	10.76
10-Apr-96	10:00	573.5														
10-Apr-96	18:20	581.83331														
11-Apr-96	2:10	589.66669														
11-Apr-96	10:15	597.75														
11-Apr-96	18:15	605.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.99	25.92	7.37	14.52	0.00	10.11

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
3/30/96	2:00	301.5	1.48	0.00	2.70	4.68	4.17	39.71	0.00	0.00
3/30/96	10:00	309.5	1.31	0.00	2.55	4.20	3.92	39.45	0.00	0.00
3/30/96	18:00	317.5								
3/31/96	2:00	325.5	1.68	0.00	2.96	5.35	4.50	38.62	0.00	0.00
3/31/96	10:00	333.5								
31-Mar-96	18:00	341.5	1.50	0.00	2.78	4.69	4.24	39.78	0.00	0.00
1-Apr-96	2:00	349.5	1.43	2.49	2.49	4.77	4.08	38.83	0.00	0.00
1-Apr-96	10:00	357.5								
1-Apr-96	18:00	365.5	1.61	0.00	2.85	5.40	4.51	37.57	0.00	0.00
2-Apr-96	2:00	373.5	1.29	2.42	4.71	4.08	4.08	37.39	0.00	0.00
2-Apr-96	10:00	381.5								
2-Apr-96	18:00	389.5	1.50	0.00	2.99	5.31	4.77	36.52	0.00	0.00
3-Apr-96	2:00	397.5	1.22	0.00	2.71	4.52	4.38	36.57	0.00	0.00
3-Apr-96	10:00	405.5								
3-Apr-96	17:30	413	1.23	0.00	3.58	4.52	4.87	36.23	0.00	0.00
4-Apr-96	2:00	421.5	1.17	0.00	4.34	4.37	5.22	35.42	0.00	0.00
4-Apr-96	10:00	429.5								
4-Apr-96	18:00	437.5	1.51	0.00	8.70	5.12	8.45	35.81	0.00	0.00
5-Apr-96	2:30	446	0.79	0.00	8.95	3.32	7.74	35.26	0.00	0.00
5-Apr-96	10:00	453.5								
5-Apr-96	18:00	461.5	1.75	0.00	12.98	5.33	11.00	35.40	0.00	0.00
6-Apr-96	2:00	469.5	1.60	0.00	13.76	5.14	11.18	34.63	0.00	0.00
6-Apr-96	10:00	477.5								
6-Apr-96	18:00	485.5	1.49	0.00	15.37	5.39	11.86	31.69	0.00	0.00
7-Apr-96	2:00	493.5	1.55	0.00	15.61	5.61	11.96	31.45	0.00	0.00
7-Apr-96	10:00	501.5								
7-Apr-96	18:00	509.5	1.50	0.00	14.99	5.74	11.52	32.75	0.00	0.00
8-Apr-96	2:00	517.5	1.41	0.00	14.23	5.55	10.99	32.76	0.00	0.00
8-Apr-96	10:00	525.5								
8-Apr-96	18:00	533.5	1.60	0.00	13.10	5.69	10.42	34.09	0.00	0.00
9-Apr-96	2:00	541.5								
9-Apr-96	10:00	549.5								
9-Apr-96	18:00	557.5	1.54	0.00	10.13	5.09	8.59	35.31	0.00	0.00
10-Apr-96	2:15	565.75	1.60	0.00	8.57	4.87	7.46	35.47	0.00	0.00
10-Apr-96	10:00	573.5								
10-Apr-96	18:20	581.83331								
11-Apr-96	2:10	589.66669								
11-Apr-96	10:15	597.75								
11-Apr-96	18:15	605.75	1.60	0.00	10.18	4.61	8.86	35.62	0.00	0.00

Run ID#: P960314CF

**Vessel: V-455B**

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass CFUs	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	DCW (g/L)	% Viability	Enz. Activ. FPU/mL	Enz. Digest	Contamination			pH	
												Scope	Liquid	Plate	Floor	Micro
12-Apr-96	2:10	613.66667			0.28	33.1	4.82									5.02
12-Apr-96	10:00	621.5			0.19	29.45	4.94									4.98
12-Apr-96	18:20	629.83333			0.15	34.8	5.62									4.86
13-Apr-96	2:30	638			0.15	34.3	5.52									5.03
13-Apr-96	10:00	645.5			0.12	30.5	6.38									4.84
13-Apr-96	18:00	653.5			0.13	34.4	8									4.95
14-Apr-96	2:00	661.5			0.2	36.5	7.62									5.06
14-Apr-96	10:00	669.5			0.2	32.15	8.01									4.79
14-Apr-96	18:00	677.5			0.2	31.7	9.74									4.95
15-Apr-96	2:00	685.5			0.7	34.2	4.81									5.10
15-Apr-96	10:00	693.5		8.05E+07	0.31	25.3	4.35									4.89
15-Apr-96	18:00	701.5			0.18	32.5	3.97									4.78
16-Apr-96	2:00	709.5			0.16	31.8	3.67									4.84
16-Apr-96	10:00	717.5		1.05E+08	0.1	31.35	3.08									4.78
16-Apr-96	18:00	725.5			0.17	32.75	2.79									4.90
17-Apr-96	2:00	733.5			0.01	33.1	2.42									4.91
17-Apr-96	10:00	741.5			0.13	32.65	2.11									4.92
17-Apr-96	18:00	749.5			0.13	34.3	1.85									4.95
18-Apr-96	3:00	758.5			0.07	34.5	1.62									4.94
18-Apr-96	10:00	765.5		3.80E+07												
18-Apr-96	10:30	766			0.22	38.05	1.09									7.11
18-Apr-96	18:00	773.5			0.32	34.6										4.99
19-Apr-96	2:00	781.5			0.15	34.5										5.07
19-Apr-96	10:00	789.5			0.1	35.85	1.28									5.02
19-Apr-96	18:00	797.5			0.1	34.6	1.3									5.02
20-Apr-96	2:00	805.5			0.1	36.8	1.17									4.96
20-Apr-96	10:15	813.75			0.11	38.4	1.15									4.98
20-Apr-96	18:00	821.5			0.12	37.65	1.1									4.90
21-Apr-96	2:15	829.75			0.1	39	1.06									5.06
21-Apr-96	10:00	837.5			0.13	41.9	1.04									5.06
21-Apr-96	18:00	845.5			0.11	40.6	1.04									4.85
22-Apr-96	2:00	853.5			0.13	43.4	1.11									5.04
22-Apr-96	10:00	861.5			0.12	46	1.04									5.03
22-Apr-96	18:00	869.5			0.15	43.3	1.01									4.89
23-Apr-96	2:00	877.5			0.16	46.2	1.12									4.99
23-Apr-96	10:00	885.5		1.30E+08												
23-Apr-96	10:30	886			0.17	46.6	0.97									5.05
23-Apr-96	18:00	893.5			0.17	43.9	0.96									4.85
24-Apr-96	2:00	901.5			0.15	49.7	1.03									4.96

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
12-Apr-96	2:10	613.66667		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.07	25.67	7.26	15.45	0.00	10.68
12-Apr-96	10:00	621.5														
12-Apr-96	18:20	629.83333		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.54	23.49	6.73	12.85	0.00	11.07
13-Apr-96	2:30	638		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.51	23.17	6.85	11.34	0.00	11.00
13-Apr-96	10:00	645.5														
13-Apr-96	18:00	653.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.01	23.88	7.47	8.16	0.00	9.85
14-Apr-96	2:00	661.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.78	22.92	7.09	6.37	0.00	9.43
14-Apr-96	10:00	669.5														
14-Apr-96	18:00	677.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.65	21.32	6.47	3.24	0.00	8.65
15-Apr-96	2:00	685.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.06	18.12	5.73	11.14	0.00	8.52
15-Apr-96	10:00	693.5														
15-Apr-96	18:00	701.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.24	16.88	5.57	13.06	0.00	9.42
16-Apr-96	2:00	709.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.13	16.53	5.45	13.67	0.00	9.47
16-Apr-96	10:00	717.5														
16-Apr-96	18:00	725.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.17	16.09	5.57	15.07	0.00	9.21
17-Apr-96	2:00	733.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.11	16.68	5.69	15.89	0.00	9.39
17-Apr-96	10:00	741.5														
17-Apr-96	18:00	749.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.12	17.28	5.96	16.41	0.00	9.68
18-Apr-96	3:00	758.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	17.10	5.84	16.47	0.00	9.64
18-Apr-96	10:00	765.5														
18-Apr-96	10:30	766														
18-Apr-96	18:00	773.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.62	16.54	5.83	17.80	0.00	9.30
19-Apr-96	2:00	781.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.18	17.49	6.05	18.55	0.00	9.88
19-Apr-96	10:00	789.5														
19-Apr-96	18:00	797.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.07	17.33	5.86	18.40	0.00	5.34
20-Apr-96	2:00	805.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.09	18.15	6.09	19.18	0.00	10.42
20-Apr-96	10:15	813.75														
20-Apr-96	18:00	821.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.34	19.52	6.54	20.29	0.00	10.84
21-Apr-96	2:15	829.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.39	19.74	6.54	20.23	0.00	11.06
21-Apr-96	10:00	837.5														
21-Apr-96	18:00	845.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.65	20.13	6.74	20.69	0.00	11.41
22-Apr-96	2:00	853.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.22	20.29	6.82	20.84	0.00	11.62
22-Apr-96	10:00	861.5														
22-Apr-96	18:00	869.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.51	21.10	7.06	21.29	0.00	11.64
23-Apr-96	2:00	877.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.44	21.61	7.15	21.47	0.00	11.88
23-Apr-96	10:00	885.5														
23-Apr-96	10:30	886														
23-Apr-96	18:00	893.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.44	21.02	6.95	21.25	0.00	11.22
24-Apr-96	2:00	901.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.34	21.52	7.06	21.49	0.00	11.56

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Xylitol	Succinic	Lactic	Glycerol	Acetic	EIOH	HMF	furfural
12-Apr-96	2:10	613.66667	1.56	0.00	9.50	4.30	8.30	34.33	0.00	0.00
12-Apr-96	10:00	621.5								
12-Apr-96	18:20	629.83333	1.74	0.00	11.71	4.70	10.05	35.20	0.00	0.00
13-Apr-96	2:30	638	1.77	0.00	12.84	4.75	10.95	34.96	0.00	0.00
13-Apr-96	10:00	645.5								
13-Apr-96	18:00	653.5	1.93	0.00	15.25	5.09	12.98	33.77	0.00	0.00
14-Apr-96	2:00	661.5	1.77	0.00	15.68	4.90	13.34	32.04	0.00	0.00
14-Apr-96	10:00	669.5								
14-Apr-96	18:00	677.5	1.70	0.00	18.63	5.34	16.15	34.61	0.00	0.00
15-Apr-96	2:00	685.5	1.32	0.00	9.35	9.95	8.72	32.99	0.00	0.00
15-Apr-96	10:00	693.5								
15-Apr-96	18:00	701.5	1.10	0.00	7.48	4.95	7.70	33.25	0.00	0.00
16-Apr-96	2:00	709.5	1.07	0.00	6.59	5.06	7.20	33.26	0.00	0.00
16-Apr-96	10:00	717.5								
16-Apr-96	18:00	725.5	1.02	0.00	4.97	5.19	5.98	33.39	0.00	0.00
17-Apr-96	2:00	733.5	1.04	0.00	4.32	5.29	5.47	33.76	0.00	0.00
17-Apr-96	10:00	741.5								
17-Apr-96	18:00	749.5	1.04	0.00	3.38	5.09	4.63	34.72	0.00	0.00
18-Apr-96	3:00	758.5	0.98	0.00	2.86	4.62	4.09	34.83	0.00	0.00
18-Apr-96	10:00	765.5								
18-Apr-96	10:30	766								
18-Apr-96	18:00	773.5	1.44	0.00	4.09	4.40	4.19	35.12	0.00	0.00
19-Apr-96	2:00	781.5	1.38	0.00	3.57	4.12	3.84	35.97	0.00	0.00
19-Apr-96	10:00	789.5								
19-Apr-96	18:00	797.5	1.39	0.00	2.92	3.57	3.38	36.92	0.00	0.00
20-Apr-96	2:00	805.5	1.50	0.00	2.69	3.37	3.26	37.96	0.00	0.00
20-Apr-96	10:15	813.75								
20-Apr-96	18:00	821.5	1.44	0.00	2.50	3.10	3.17	38.15	0.00	0.00
21-Apr-96	2:15	829.75	1.52	0.00	2.46	3.03	3.21	40.69	0.00	0.00
21-Apr-96	10:00	837.5								
21-Apr-96	18:00	845.5	1.54	0.00	2.36	2.90	3.19	43.23	0.00	0.00
22-Apr-96	2:00	853.5	1.55	0.00	2.33	2.85	3.17	44.81	0.00	0.00
22-Apr-96	10:00	861.5								
22-Apr-96	18:00	869.5	1.55	0.00	2.13	2.54	2.99	46.66	0.00	0.00
23-Apr-96	2:00	877.5	1.56	0.00	2.14	2.58	3.05	47.34	0.00	0.00
23-Apr-96	10:00	885.5								
23-Apr-96	10:30	886								
23-Apr-96	18:00	893.5	1.60	0.00	2.06	2.46	3.07	49.41	0.00	0.00
24-Apr-96	2:00	901.5	1.51	0.00	2.06	2.45	3.15	49.13	0.00	0.00

Run ID#: P960314CF

**Vessel: V-455B**

[illegible]

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt. (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
24-Apr-96	10:00	909.5														
24-Apr-96	18:00	917.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.41	21.16	6.94	21.26	0.00	11.27
25-Apr-96	2:00	925.5														
25-Apr-96	10:00	933.5														
25-Apr-96	18:00	941.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.42	21.70	7.16	21.99	0.00	11.71
26-Apr-96	2:00	949.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.33	21.20	7.07	21.81	0.00	11.70
26-Apr-96	10:00	957.5														
26-Apr-96	18:00	965.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	20.18	6.89	21.13	0.00	11.43
27-Apr-96	2:00	973.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	19.41	6.68	19.91	0.00	11.23
27-Apr-96	10:15	981.75														
27-Apr-96	18:00	989.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.30	18.10	6.55	16.11	0.00	11.10
28-Apr-96	2:00	997.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.20	17.66	6.50	14.24	0.00	10.96
28-Apr-96	10:00	1005.5														
28-Apr-96	18:00	1013.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.24	16.33	6.12	8.11	0.00	10.10
29-Apr-96	2:00	1021.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.91	15.95	6.02	9.40	0.00	9.94
29-Apr-96	9:40	1029.1667		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.69	16.71	6.17	8.27	0.00	10.08
29-Apr-96	18:00	1037.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	7.09	18.56	6.33	8.15	0.00	10.40

Run start data 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)								
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural
24-Apr-96	10:00	909.5								
24-Apr-96	18:00	917.5	1.58	0.00	2.11	2.53	3.22	50.50	0.00	0.00
25-Apr-96	2:00	925.5								
25-Apr-96	10:00	933.5								
25-Apr-96	18:00	941.5	1.50	0.00	2.16	2.47	3.24	49.46	0.00	0.00
26-Apr-96	2:00	949.5	1.52	0.00	2.55	2.92	3.72	50.66	0.00	0.00
26-Apr-96	10:00	957.5								
26-Apr-96	18:00	965.5	1.46	0.00	2.86	2.62	3.68	48.52	0.00	0.00
27-Apr-96	2:00	973.5	1.53	0.00	3.50	2.70	4.16	49.85	0.00	0.00
27-Apr-96	10:15	981.75								
27-Apr-96	18:00	989.5	1.46	0.69	5.85	2.89	5.79	49.74	0.00	0.00
28-Apr-96	2:00	997.5	1.58	0.80	7.50	3.12	7.02	51.54	0.00	0.00
28-Apr-96	10:00	1005.5								
28-Apr-96	18:00	1013.5	2.95	0.00	9.82	3.17	8.60	50.75	0.00	0.00
29-Apr-96	2:00	1021.5	3.07	1.01	10.87	3.19	9.46	49.80	0.00	0.00
29-Apr-96	9:40	1029.1667	1.57	1.11	11.75	3.12	10.08	47.13	0.00	0.00
29-Apr-96	18:00	1037.5	1.61	1.06	12.61	3.27	10.78	44.58	0.00	0.00

Run ID#: P960314CF

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass CFUs	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	DCW (g/L)	% Viability	Enz. Activ. FPU/mL	Enz. Digest	Contamination			pH	
												Scope	Liquid	Plate	Floor	Micro
3/19/96	18:00	53.5			0.52	42.9	1.34									5.10
3/20/96	2:20	61.83333333			0.44	43.1	1.3									5.03
3/20/96	7:45	67.25		1.03E+08									clean			
3/20/96	10:00	69.5			0.43	42.55	1.32									5.04
3/20/96	18:00	77.5			0.37	45.6	1.3									5.01
3/21/96	2:00	85.5			0.358	49.1	1.28									5.05
3/21/96	10:00	93.5		1.03E+08	0.28	43.5	1.23						clean			4.99
3/21/96	18:00	101.5			0.16	41.5	1.23									5.01
3/22/96	2:00	109.5			0.328	43.25	1.19									4.96
3/22/96	10:00	117.5		8.55E+07	0.35	41.1	1.18						clean			5.07
3/22/96	18:00	125.5			0.27	39.4	1.18									4.88
3/23/96	2:00	133.5			0.38	43.5	1.18									5.01
3/23/96	10:00	141.5			0.42	38.9	1.17									4.90
3/23/96	18:00	149.5			0.35	43.6	1.17									4.90
3/24/96	2:00	157.5			0.36		1.13									4.96
3/24/96	10:00	165.5			0.34	38.2	1.11									4.91
3/24/96	18:00	173.5			0.29	38.8	1.09									4.92
3/25/96	2:00	181.5			0.4	42.6	1.11									4.88
3/25/96	10:00	189.5		7.10E+07	0.39	39.1	1.09						rods			4.94
3/25/96	18:00	197.5			0.45	39.7	1.1									4.91
3/26/96	2:00	205.5			0.49	41.3	1.12									4.85
3/26/96	10:00	213.5		5.80E+07	0.83	38	1.14						rods			5.05
3/26/96	18:00	221.5			0.34	40.8	1.1									4.86
3/27/96	2:00	229.5			0.41	38.1	1.11									4.85
3/27/96	10:00	237.5		5.35E+07	0.448	38.85	1.15						rods			4.94
3/27/96	18:00	245.5			0.386	37.35	1.16									4.91
3/28/96	2:00	253.5			0.33	37.5	1.21									4.99
3/28/96	9:00	260.5		5.80E+07	0.36	38.8	1.21						rods			4.99
3/28/96	18:00	269.5			0.3	38.2	1.24									5.01
3/29/96	2:00	277.5			0.29	38.7	1.33									5.04
3/29/96	9:00	284.5		4.95E+07									rods			
3/29/96	10:00	285.5			0.29	39.4	1.31									5.03
3/29/96	18:00	293.5			0.23	37.8	1.31									4.94
3/30/96	2:00	301.5			0.27	36.4	1.34									5.01
3/30/96	10:00	309.5			0.214	39.5	1.39									5.05
3/30/96	18:00	317.5			0.3	36.9	1.34									5.10
3/31/96	2:00	325.5			0.28	36.9	1.33									5.11
3/31/96	10:00	333.5			0.32	38.45	1.35									5.07
3/31/96	18:00	341.5			0.28	36.65	1.29									5.10

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
3/19/96	18:00	53.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.64	23.97	7.76	25.36	0.00	9.62
3/20/96	2:20	61.83333333		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.48	22.98	7.91	25.40	0.00	9.32
3/20/96	7:45	67.25														
3/20/96	10:00	69.5														
3/20/96	18:00	77.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.66	22.00	7.97	25.34	0.00	9.07
3/21/96	2:00	85.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.31	22.01	7.95	25.23	0.00	9.05
3/21/96	10:00	93.5														
3/21/96	18:00	101.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.52	23.08	8.25	25.28	0.00	8.78
3/22/96	2:00	109.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.19	22.72	8.04	24.66	0.00	8.44
3/22/96	10:00	117.5														
3/22/96	18:00	125.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.28	23.23	7.91	24.32	1.03	10.04
3/23/96	2:00	133.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.19	23.06	7.91	24.22	0.00	9.89
3/23/96	10:00	141.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.26	23.36	8.12	24.69	0.00	8.13
3/23/96	18:00	149.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.36	24.27	8.54	25.74	0.00	8.51
3/24/96	2:00	157.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.16	23.91	8.24	24.98	0.00	8.23
3/24/96	10:00	165.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.11	23.13	8.06	24.40	0.00	6.34
3/24/96	18:00	173.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.16	23.98	8.32	25.13	0.00	7.60
3/25/96	2:00	181.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.01	22.88	7.99	24.15	0.00	7.40
3/25/96	10:00	189.5														
3/25/96	18:00	197.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.08	23.10	8.04	24.87	0.00	0.00
3/26/96	2:00	205.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.01	22.87	8.02	24.68	0.00	0.00
3/26/96	10:00	213.5														
3/26/96	18:00	221.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.18	23.12	7.95	24.50	0.00	7.59
3/27/96	2:00	229.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.97	23.04	7.92	24.25	0.00	7.38
3/27/96	10:00	237.5														
3/27/96	18:00	245.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.84	22.28	7.26	23.19	0.00	6.55
3/28/96	2:00	253.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.81	23.64	7.58	24.20	0.00	7.30
3/28/96	9:00	260.5														
3/28/96	18:00	269.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.89	23.54	7.31	23.61	0.00	7.67
3/29/96	2:00	277.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.87	23.68	7.22	23.46	0.00	7.78
3/29/96	9:00	284.5														
3/29/96	10:00	285.5														
3/29/96	18:00	293.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.99	24.61	7.29	23.80	0.00	8.15
3/30/96	2:00	301.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.94	25.30	7.36	24.05	0.00	8.44
3/30/96	10:00	309.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.81	25.43	7.32	23.92	0.00	8.39
3/30/96	18:00	317.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	2.21	25.40	7.39	23.67	0.00	8.29
3/31/96	2:00	325.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.86	25.98	7.49	24.19	0.00	8.51
3/31/96	10:00	333.5														
3/31/96	18:00	341.5														

Run ID#: P960314CF

Date	Time	Run time (h)	Sugar								GC Ethanol	Acid Sol. Lignin (g/L)
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural		
3/19/96	18:00	53.5	0.00	0.00	2.16	3.80	3.07		0.00	0.00	0.00	0.00
3/20/96	2:20	61.83333333	0.00	0.00	2.05	3.72	2.96		0.00	0.00	0.00	0.00
3/20/96	7:45	67.25										
3/20/96	10:00	69.5										
3/20/96	18:00	77.5	1.74	0.00	1.85	3.52	3.12	44.34	0.00	0.00	0.00	0.00
3/21/96	2:00	85.5	1.89	0.00	2.00	3.85	3.35	45.22	0.00	0.00	0.00	0.00
3/21/96	10:00	93.5										
3/21/96	18:00	101.5	0.00	0.00	2.49	4.66	4.05		0.00	0.00	0.00	0.00
3/22/96	2:00	109.5	0.00	0.00	2.31	4.38	3.83		0.00	0.00	0.00	0.00
3/22/96	10:00	117.5										
3/22/96	18:00	125.5	1.34	0.00	1.80	3.62	3.44	43.34	0.00	0.00	0.00	0.00
3/23/96	2:00	133.5	1.51	0.00	2.03	4.03	3.79	42.02	0.00	0.00	0.00	0.00
3/23/96	10:00	141.5	1.91	0.00	2.58	5.06	4.55	42.67	0.00	0.00	0.00	0.00
3/23/96	18:00	149.5	1.88	0.00	2.54	5.05	4.67	42.82	0.00	0.00	0.00	0.00
3/24/96	2:00	157.5	1.93	0.00	2.57	5.18	4.71	42.49	0.00	0.00	0.00	0.00
3/24/96	10:00	165.5	1.90	0.00	3.17	6.09	5.68	43.64	0.00	0.00	0.00	0.00
3/24/96	18:00	173.5	1.94	0.00	3.16	6.12	5.79	43.04	0.00	0.00	0.00	0.00
3/25/96	2:00	181.5	1.93	0.00	3.12	6.11	5.84	42.94	0.00	0.00	0.00	0.00
3/25/96	10:00	189.5										
3/25/96	18:00	197.5	2.03	0.00	2.63	5.33	5.21	41.96	0.00	0.00	0.00	0.00
3/26/96	2:00	205.5	1.74	0.00	2.10	4.52	4.72	40.94	0.00	0.00	0.00	0.00
3/26/96	10:00	213.5										
3/26/96	18:00	221.5	2.08	0.00	2.66	5.55	5.33	43.15	0.00	0.00	0.00	0.00
3/27/96	2:00	229.5	1.78	0.00	2.10	4.67	4.82	42.67	0.00	0.00	0.00	0.00
3/27/96	10:00	237.5										
3/27/96	18:00	245.5	1.59	0.00	2.02	4.24	4.50	40.70	0.00	0.00	0.00	0.00
3/28/96	2:00	253.5	1.74	0.00	2.37	4.70	4.83	41.14	0.00	0.00	0.00	0.00
3/28/96	9:00	260.5										
3/28/96	18:00	269.5	1.57	0.00	2.29	4.31	4.37	40.89	0.00	0.00	0.00	0.00
3/29/96	2:00	277.5	1.51	0.00	2.33	4.21	4.23	41.72	0.00	0.00	0.00	0.00
3/29/96	9:00	284.5										
3/29/96	10:00	285.5										
3/29/96	18:00	293.5	1.88	0.00	3.13	5.32	4.89	41.91	0.00	0.00	0.00	0.00
3/30/96	2:00	301.5	1.38	0.00	2.38	4.00	3.91	40.67	0.00	0.00	0.00	0.00
3/30/96	10:00	309.5	1.39	0.00	2.37	3.98	3.87	40.57	0.00	0.00	0.00	0.00
3/30/96	18:00	317.5	1.82	0.00	3.05	5.10	4.55	40.20	0.00	0.00	0.00	0.00
3/31/96	2:00	325.5	1.25	0.00	2.14	3.83	3.58	39.64	0.00	0.00	0.00	0.00
3/31/96	10:00	333.5										
3/31/96	18:00	341.5										



Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
4/1/96	2:00	349.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.89	25.95	7.43	23.92	0.00	8.22
4/1/96	10:00	357.5														
4/1/96	18:00	365.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.89	24.87	7.35	23.52	0.00	8.43
4/2/96	2:00	373.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.97	25.41	7.56	23.98	0.00	8.76
4/2/96	10:00	381.5														
4/2/96	18:00	389.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.89	24.86	7.53	23.17	0.00	9.11
4/3/96	2:00	397.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.86	25.47	7.69	23.83	0.00	9.16
4/3/96	10:00	405.5		19.93%		0.00%	93.80	22.05%	0.00%	297.91	0.17	23.81	5.32	20.99	0.00	3.15
3-Apr-96	10:10	405.6666667		19.29%		0.00%	94.20	23.33%	0.00%	302.88	0.15	24.39	5.73	21.54	0.00	3.21
3-Apr-96	10:20	405.8333333		19.59%		0.00%	101.90	21.97%	0.00%	334.00	0.17	23.50	5.49	20.96	0.00	3.22
4/3/96	17:30	413		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.80	25.15	7.54	20.31	0.00	9.38
4/4/96	2:00	421.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.59	24.57	7.32	18.35	0.00	9.29
4/4/96	10:00	429.5														
4/4/96	18:00	437.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.98	23.54	6.98	14.41	0.00	9.51
4/5/96	2:30	446		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.75	22.93	6.65	12.08	0.00	9.54
4/5/96	10:00	453.5														
4/5/96	18:00	461.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.67	21.82	6.19	8.52	0.00	9.31
4/6/96	2:00	469.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.66	21.92	6.10	7.21	0.00	9.51
4/6/96	10:00	477.5														
4/6/96	18:00	485.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.72	21.78	5.98	5.45	0.00	9.34
4/7/96	2:00	493.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.69	22.16	6.09	4.74	0.00	9.46
4/7/96	10:00	501.5														
4/7/96	18:00	509.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.63	22.28	6.11	4.17	0.00	10.17
4/8/96	2:00	517.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.56	22.69	6.18	4.30	0.00	9.86
4/8/96	10:00	525.5														
4/8/96	18:00	533.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.82	22.88	6.34	5.25	0.00	9.49
4/9/96	2:00	541.5														
4/9/96	10:00	549.5														
9-Apr-96	18:00	557.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.61	23.92	6.42	7.16	0.00	9.53
10-Apr-96	2:15	565.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.77	24.79	6.64	9.72	0.00	16.10
10-Apr-96	10:00	573.5														
10-Apr-96	18:20	581.833313														
11-Apr-96	2:10	589.666687														
11-Apr-96	10:15	597.75														
11-Apr-96	18:15	605.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.64	23.96	6.78	7.82	0.00	9.85
12-Apr-96	2:10	613.6666667		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.59	23.92	6.74	7.34	0.00	9.91
12-Apr-96	10:00	621.5														
12-Apr-96	18:20	629.8333333		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.37	22.24	6.20	4.76	0.00	10.54
13-Apr-96	2:30	638		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.37	21.75	6.13	2.27	0.89	10.32

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)									GC Ethanol	Acid Sol. Lignin (g/L)
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural		
4/1/96	2:00	349.5	1.28	0.00	2.19	3.86	3.65	40.62	0.00	0.00	0.00	0.00
4/1/96	10:00	357.5										
4/1/96	18:00	365.5	1.17	0.00	2.11	3.84	3.65	39.65	0.00	0.00	0.00	0.00
4/2/96	2:00	373.5	1.10	0.00	2.11	3.73	3.67	39.00	0.00	0.00	0.00	0.00
4/2/96	10:00	381.5										
4/2/96	18:00	389.5	1.01	0.00	2.74	3.65	4.28	37.98	0.00	0.00	0.00	0.00
4/3/96	2:00	397.5	1.03	0.00	2.49	3.72	4.02	38.52	0.00	0.00	0.00	0.00
4/3/96	10:00	405.5	0.00	0.79	4.26	5.02	5.72	37.12	0.00	0.00	0.00	0.00
3-Apr-96	10:10	405.6666667	0.00	0.88	4.39	5.16	6.21	38.63	0.00	0.00	36.26	9.48
3-Apr-96	10:20	405.8333333	0.00	0.84	4.29	5.18	5.99	38.81	0.00	0.00	36.87	9.97
4/3/96	17:30	413	1.27	0.00	4.31	4.27	5.67	37.13	0.00	0.00	36.25	15.17
4/4/96	2:00	421.5	1.24	0.00	5.96	4.21	6.73	36.04	0.00	0.00	0.00	0.00
4/4/96	10:00	429.5										
4/4/96	18:00	437.5	1.19	0.00	9.99	4.04	9.26	35.90	0.00	0.00	0.00	0.00
4/5/96	2:30	446	1.25	0.00	12.00	4.09	10.53	35.45	0.00	0.00	0.00	0.00
4/5/96	10:00	453.5										
4/5/96	18:00	461.5	2.61	0.00	15.87	5.06	13.40	35.44	0.00	0.00	0.00	0.00
4/6/96	2:00	469.5	2.73	0.00	17.05	5.24	14.13	34.93	0.00	0.00	0.00	0.00
4/6/96	10:00	477.5										
4/6/96	18:00	485.5	2.79	0.00	18.76	5.33	15.04	32.49	0.00	0.00	0.00	0.00
4/7/96	2:00	493.5	2.86	0.00	19.74	5.60	15.63	32.25	0.00	0.00	0.00	0.00
4/7/96	10:00	501.5										
4/7/96	18:00	509.5	2.78	0.00	19.89	5.97	15.82	32.64	0.00	0.00	0.00	0.00
4/8/96	2:00	517.5	2.62	0.00	19.47	5.70	15.26	32.01	0.00	0.00	0.00	0.00
4/8/96	10:00	525.5										
4/8/96	18:00	533.5	2.63	0.00	19.96	6.29	15.85	32.98	0.00	0.00	0.00	0.00
4/9/96	2:00	541.5										
4/9/96	10:00	549.5										
9-Apr-96	18:00	557.5	1.92	0.00	17.86	6.15	14.72	33.52	0.00	0.00	0.00	0.00
10-Apr-96	2:15	565.75	1.79	0.00	15.66	5.70	13.13	34.28	0.00	0.00	0.00	0.00
10-Apr-96	10:00	573.5										
10-Apr-96	18:20	581.833313										
11-Apr-96	2:10	589.666687										
11-Apr-96	10:15	597.75										
11-Apr-96	18:15	605.75	1.77	0.00	16.91	5.33	14.11	33.71	0.00	0.00	0.00	0.00
12-Apr-96	2:10	613.6666667	1.81	0.00	16.86	5.34	14.27	34.41	0.00	0.00	0.00	0.00
12-Apr-96	10:00	621.5										
12-Apr-96	18:20	629.8333333	2.95	0.00	19.46	5.52	16.15	33.82	0.00	0.00	0.00	0.00
13-Apr-96	2:30	638	3.22	0.00	20.92	5.60	17.23	33.65	0.00	0.00	0.00	0.00

17-Mar-96 Time 12:30

Run Name: CRADA Task 4

Run ID#: P960314CF

## PDU Analytical Results

**Vessel:** Y-455C

Date	Time	Run time (h)	O.D. 600 nm	Cell Mass CFUs	YSI Gluc (g/L)	YSI EtOH (g/L)	YSI Lactate (g/L)	DCW (g/L)	% Viability	Enz. Activ FPU/mL	Enz. Digest	Contamination			pH	
												Scope	Liquid	Plate	Floor	Micro
13-Apr-96	10:00	645.5			0.11	31.45	7.77									4.87
13-Apr-96	18:00	653.5			0.13	31.6	9.8									5.02
14-Apr-96	2:00	661.5			0.19	33.9	8.84									5.08
14-Apr-96	10:00	669.5			0.2	27.9	8.47									4.81
14-Apr-96	18:00	677.5			0.14	29	10.05									5.04
15-Apr-96	2:00	685.5			0.15	33.5	7.77									5.06
15-Apr-96	10:00	693.5		8.70E+07	0.15	25.2	5.89									4.91
15-Apr-96	18:00	701.5			0.16	31.95	5.76									4.89
16-Apr-96	2:00	709.5			0.11	31.7	6.88									4.74
16-Apr-96	10:00	717.5		9.05E+07	0.1	30.4	5.59									4.74
16-Apr-96	18:00	725.5			0.16	31.45	5.21									4.88
17-Apr-96	2:00	733.5			0.01	31.8	5.06									4.87
17-Apr-96	10:00	741.5			0.08	31.65	4.35									4.89
17-Apr-96	18:00	749.5			0.08	33.2	3.8									4.92
18-Apr-96	3:00	758.5			0.07	34.7	2.67									4.84
18-Apr-96	10:00	765.5		9.45E+07												
18-Apr-96	10:30	766			0.08	35.7	2.72									5.00
18-Apr-96	18:00	773.5			0.13	33.6										4.89
19-Apr-96	2:00	781.5			0.12	32.5										5.02
19-Apr-96	10:00	789.5			0.08	33.5	2.27									5.01
19-Apr-96	18:00	797.5			0.1	33.7	2.24									4.99
20-Apr-96	2:00	805.5			0.1	33.1	1.93									4.91
20-Apr-96	10:15	813.75			0.08	35.25	1.78									4.96
20-Apr-96	18:00	821.5			0.08	35.15	1.57									4.87
21-Apr-96	2:15	829.75			0.08	36.5	1.45									5.00
21-Apr-96	10:00	837.5			0.1	37.9	1.37									5.05
21-Apr-96	18:00	845.5			0.09	36.8	1.32									4.86
22-Apr-96	2:00	853.5			0.05	38.9	1.45									5.06
22-Apr-96	10:00	861.5			0.09	41.65	1.24									5.05
22-Apr-96	18:00	869.5			0.1	38.5	1.19									4.92
23-Apr-96	2:00	877.5			0	42.7	1.24									5.02
23-Apr-96	10:00	885.5		1.21E+08												
23-Apr-96	10:30	886			0.12	42.8	1.1									5.07
23-Apr-96	18:00	893.5			0.13	42.75	1.07									4.86
24-Apr-96	2:00	901.5			0.11	45.1	1.18									5.02
24-Apr-96	10:00	909.5		1.05E+08	0.11	43.5	1.02									5.23
24-Apr-96	18:00	917.5			0.18	43.35	1.01									5.04
25-Apr-96	2:00	925.5			0.14	47.8	1.25									5.10
25-Apr-96	10:00	933.5		2.95E+07	0.11	45.1	1									5.07

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
13-Apr-96	10:00	645.5														
13-Apr-96	18:00	653.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.93	22.64	6.69	2.04	0.00	9.07
14-Apr-96	2:00	661.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.67	21.35	6.06	1.51	0.00	8.34
14-Apr-96	10:00	669.5														
14-Apr-96	18:00	677.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.55	19.92	5.14	1.13	0.00	7.44
15-Apr-96	2:00	685.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.54	20.10	5.17	1.34	0.92	7.61
15-Apr-96	10:00	693.5														
15-Apr-96	18:00	701.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.34	17.75	5.38	7.90	0.00	9.09
16-Apr-96	2:00	709.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.15	17.64	5.33	8.07	0.00	9.16
16-Apr-96	10:00	717.5														
16-Apr-96	18:00	725.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.25	16.14	5.34	9.36	8.74	8.74
17-Apr-96	2:00	733.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.16	16.05	5.39	10.52	0.00	8.84
17-Apr-96	10:00	741.5														
17-Apr-96	18:00	749.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.10	16.12	5.68	11.74	0.00	8.90
18-Apr-96	3:00	758.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	16.52	5.71	14.08	0.00	3.20
18-Apr-96	10:00	765.5														
18-Apr-96	10:30	766														
18-Apr-96	18:00	773.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.18	16.70	6.05	15.10	0.00	9.18
19-Apr-96	2:00	781.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.05	16.52	5.94	15.06	0.00	9.16
19-Apr-96	10:00	789.5														
19-Apr-96	18:00	797.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.99	15.77	5.71	15.56	0.00	5.80
20-Apr-96	2:00	805.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	16.58	5.93	16.61	0.00	9.54
20-Apr-96	10:15	813.75														
20-Apr-96	18:00	821.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.14	17.20	6.17	17.69	0.00	9.64
21-Apr-96	2:15	829.75		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	17.78	6.31	18.36	0.00	10.07
21-Apr-96	10:00	837.5														
21-Apr-96	18:00	845.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.08	18.08	6.44	18.87	0.00	10.23
22-Apr-96	2:00	853.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	18.15	6.46	18.98	0.00	10.39
22-Apr-96	10:00	861.5														
22-Apr-96	18:00	869.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.24	18.71	6.82	19.79	0.00	10.68
23-Apr-96	2:00	877.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.69	18.53	6.69	18.94	0.00	10.02
23-Apr-96	10:00	885.5														
23-Apr-96	10:30	886														
23-Apr-96	18:00	893.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.28	18.84	6.71	19.94	0.00	10.44
24-Apr-96	2:00	901.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	3.66	19.52	7.01	19.56	0.00	8.76
24-Apr-96	10:00	909.5														
24-Apr-96	18:00	917.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.27	19.32	6.92	20.42	0.00	10.54
25-Apr-96	2:00	925.5														
25-Apr-96	10:00	933.5														

Run ID#: P960314CF

Date	Time	Run time (h)	GC								GC Ethanol	Acid Sol. Lignin (g/L)
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	furfural		
13-Apr-96	10:00	645.5										
13-Apr-96	18:00	653.5	3.45	0.00	22.40	5.74	18.49	31.22	0.00	0.00	0.00	0.00
14-Apr-96	2:00	661.5	3.57	0.00	22.98	5.81	18.95	31.46	0.00	0.00	0.00	0.00
14-Apr-96	10:00	669.5										
14-Apr-96	18:00	677.5	3.64	0.00	24.17	5.82	20.17	32.26	0.00	0.00	0.00	0.00
15-Apr-96	2:00	685.5	3.38	0.00	22.57	5.49	19.07	31.90	0.00	0.00	0.00	0.00
15-Apr-96	10:00	693.5										
15-Apr-96	18:00	701.5	1.46	0.00	13.88	5.38	12.53	32.73	0.00	0.00	0.00	0.00
16-Apr-96	2:00	709.5	1.45	0.00	13.38	5.55	12.31	32.57	0.00	0.00	0.00	0.00
16-Apr-96	10:00	717.5										
16-Apr-96	18:00	725.5	1.33	0.00	11.16	5.55	11.24	32.26	0.00	0.00	0.00	0.00
17-Apr-96	2:00	733.5	1.24	0.00	9.74	5.50	10.17	32.46	0.00	0.00	0.00	0.00
17-Apr-96	10:00	741.5										
17-Apr-96	18:00	749.5	1.19	0.00	7.55	5.31	8.48	33.12	0.00	0.00	0.00	0.00
18-Apr-96	3:00	758.5	1.10	0.00	5.12	4.98	6.29	34.03	0.00	0.00	0.00	0.00
18-Apr-96	10:00	765.5										
18-Apr-96	10:30	766										
18-Apr-96	18:00	773.5	1.28	0.00	5.51	5.39	6.95	34.24	0.00	0.00	0.00	0.00
19-Apr-96	2:00	781.5	1.29	0.00	5.24	5.18	6.62	33.78	0.00	0.00	0.00	0.00
19-Apr-96	10:00	789.5										
19-Apr-96	18:00	797.5	1.38	0.00	4.39	4.54	5.47	34.41	0.00	0.00	0.00	0.00
20-Apr-96	2:00	805.5	1.40	0.00	3.86	4.18	4.82	35.65	0.00	0.00	0.00	0.00
20-Apr-96	10:15	813.75										
20-Apr-96	18:00	821.5	1.53	0.00	3.69	4.15	4.56	35.92	0.00	0.00	0.00	0.00
21-Apr-96	2:15	829.75	1.53	0.00	3.37	3.85	4.26	37.33	0.00	0.00	0.00	0.00
21-Apr-96	10:00	837.5										
21-Apr-96	18:00	845.5	1.60	0.00	3.06	3.60	3.99	39.00	0.00	0.00	0.00	0.00
22-Apr-96	2:00	853.5	1.62	0.00	2.97	3.52	3.88	39.89	0.00	0.00	0.00	0.00
22-Apr-96	10:00	861.5										
22-Apr-96	18:00	869.5	1.60	0.00	2.66	3.18	3.61	42.21	0.00	0.00	0.00	0.00
23-Apr-96	2:00	877.5	1.58	0.00	2.49	2.98	3.47	43.10	0.00	0.00	0.00	0.00
23-Apr-96	10:00	885.5										
23-Apr-96	10:30	886										
23-Apr-96	18:00	893.5	1.62	0.00	2.39	2.91	3.45	45.68	0.00	0.00	0.00	0.00
24-Apr-96	2:00	901.5	1.64	0.00	2.31	2.82	3.38	45.61	0.00	0.00	0.00	0.00
24-Apr-96	10:00	909.5										
24-Apr-96	18:00	917.5	1.67	0.00	2.32	2.86	3.49	47.59	0.00	0.00	0.00	0.00
25-Apr-96	2:00	925.5										
25-Apr-96	10:00	933.5										

Run ID#: P960314CF

**Vessel: V-455C**

[illegible]

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)	Chem	Total Solids			Washed Solids		Acid Conc. (%)	Sample Wt (g)	HPLC (g/L)					
				Oven (%)	IR (%)	TDS (%)	Weight (g)	TS (%)			Glucose	Xylose	Gal.	Arab.	Man.	Cello.
25-Apr-96	18:00	941.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.27	20.54	7.24	21.06	0.00	10.99
26-Apr-96	2:00	949.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	20.01	7.02	20.62	0.00	10.79
26-Apr-96	10:00	957.5														
26-Apr-96	10:30	958		18.55%		0.00%	105.10	16.86%	0.00%	386.24	0.68	19.70	4.56	18.94	0.00	2.23
26-Apr-96	18:00	965.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	19.55	6.86	18.53	0.00	10.69
27-Apr-96	2:00	973.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.00	19.33	6.84	16.87	0.00	10.77
27-Apr-96	10:15	981.75														
27-Apr-96	18:00	989.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.85	18.04	6.54	11.10	0.00	10.62
28-Apr-96	2:00	997.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.81	17.60	6.37	8.45	0.00	10.55
28-Apr-96	10:00	1005.5														
28-Apr-96	18:00	1013.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.00	16.17	5.63	4.12	0.00	9.78
29-Apr-96	2:00	1021.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	0.98	15.86	5.48	2.52	0.00	9.59
29-Apr-96	9:40	1029.166667		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.35	16.17	5.76	2.67	0.00	9.78
29-Apr-96	18:00	1037.5		0.00%		0.00%	0.00	0.00%	0.00%	0.00	1.87	16.29	5.77	2.56	0.00	9.85

Run start date 17-Mar-96

Run Name: CRADA Task 4

Run ID#: P960314CF

Date	Time	Run time (h)									GC Ethanol	Acid Sol. Lignin (g/L)
			Xylitol	Succinic	Lactic	Glycerol	Acetic	EtOH	HMF	lurfural		
25-Apr-96	18:00	941.5	1.64	0.00	2.48	2.72	3.69	48.11	0.00	0.00	0.00	0.00
26-Apr-96	2:00	949.5	1.58	0.00	2.84	2.88	4.14	48.62	0.00	0.00	0.00	0.00
26-Apr-96	10:00	957.5										
26-Apr-96	10:30	958	1.80	0.00	3.51	2.94	4.59	49.50	0.00	0.00	48.25	11.46
26-Apr-96	18:00	965.5	1.77	0.00	4.33	2.84	5.17	49.98	0.00	0.00	0.00	0.00
27-Apr-96	2:00	973.5	1.75	0.00	5.68	2.83	6.19	49.17	0.00	0.00	0.00	0.00
27-Apr-96	10:15	981.75										
27-Apr-96	18:00	989.5	1.84	0.85	9.74	3.29	9.16	50.55	0.00	0.00	0.00	0.00
28-Apr-96	2:00	997.5	1.87	0.89	11.85	3.21	10.67	50.65	0.00	0.00	0.00	0.00
28-Apr-96	10:00	1005.5										
28-Apr-96	18:00	1013.5	3.64	1.04	15.34	3.36	13.47	48.49	0.00	0.00	0.00	0.00
29-Apr-96	2:00	1021.5	3.70	1.01	16.43	3.39	14.36	47.41	0.00	0.00	0.00	0.00
29-Apr-96	9:40	1029.166667	2.00	1.24	16.89	3.50	14.68	48.77	0.00	0.00	0.00	0.00
29-Apr-96	18:00	1037.5	1.90	1.26	16.69	3.46	14.46	47.02	0.00	0.00	0.00	0.00



Cellulose/Starch Conversion:	82.4%
Xylan Conversion:	87.4%
Overall C6-Sugar Conversion:	61.9%
Overall C5-Sugar Conversion:	17.9%
Ethanol Process Yield (% theor):	36.8%
Ethanol Metabolic Yield (% theor):	76.3%

Carbon Out											Conversion	Yield	Std.
In Solids					In Liquid					Total	(In-Out)/In	g product/ 100 g C6/C5 cons	Deviation
(% dry wt)	(C-mole/h)	(% Total Out)			(g/L)	(C-mole/h)	(% Total Out)			(C-mole/h)	(%)		
SD Comp. Sqr. Error					SD Comp. Sqr. Error					Sqr. Error			
30.39	65.463	54.8	0.8	32.9	3.19	9.654		0.0	0.710	9.654	0.710		
1.94	4.179	16.7	0.1	0.2	18.75	53.908	45.2	0.2	22.132	119.371	55.025	68.09	1.5
0.00	0.002	100.0	0.0	0.0	7.24	20.816	83.3	0.2	3.664	24.995	3.852	17.35	1.3
8.94	19.258	14.3	0.2	2.7	0.00	0.000	0.0			0.002	0.000		
3.54	7.625	10.3	0.2	0.6	40.19	115.650	85.7	0.5	102.491	134.808	105.217	12.01	6.9
					23.11	66.444	89.7	0.6	36.539	74.089	37.168	26.71	3.7
28.52	88.159	72.6	0.8	60.8	8.11	33.460	27.5	2.0	76.523	121.619	137.343	-35.64	15.3
					36.46	136.644		0.4	142.482	136.644	142.482	35.46	6.17
					5.00	17.226		1.0	14.106	17.226	14.106	4.86	1.29
						68.322			35.621	68.322	35.621	33.87	5.90
					5.12	14.398		0.1	1.626	14.398	1.626	4.98	0.87
					2.77	7.964		0.3	1.430	7.964	1.430	2.69	0.57
					2.71	7.792		0.1	0.498	7.792	0.498	2.64	0.46
					0.84	2.456		0.1	0.067	2.456	0.067	0.82	0.15
					0.00	0.000		0.0		0.000	0.000	0.00	0.00
73.33	184.685	25.0			554.634	75.0			739.320	535.144		85.32	8.712476



Cellulose/Starch Conversion:	85.1%
Xylan Conversion:	96.1%
Overall C6-Sugar Conversion:	66.9%
Overall C5-Sugar Conversion:	31.2%
Ethanol Process Yield (% theor):	41.1%
Ethanol Metabolic Yield (% theor):	73.0%

Carbon Out											Conversion	Yield	Sid.
in Solids			in Liquid			Total					(In-Out)/In	g product/ 100 g C6/C5 cons	Deviation
(% dry wt)	(C-mole/h)	(% Total Out)	(g/L)	(C-mole/h)	(% Total Out)	(C-mole/h)					(%)		
SD Comp. Sqr. Error					SD Comp. Sqr. Error					Sqr. Error			
39.25	55.662	62.2	0.8	27.2	2.23	6.920	0.0	0.537	6.920	0.537			
0.80	1.135	5.6	0.1	0.0	17.32	51.056	47.8	0.2	106.718	56.207	71.53		1.5
0.00	0.001	100.0	0.0	0.0	6.51	19.190	94.4	0.2	20.325	4.527	32.93		1.5
4.26	6.041	5.5	0.2	0.4	0.00	0.000	0.0		0.001	0.000			
1.32	1.872	2.9	0.2	0.1	35.37	104.264	94.5	0.5	110.306	122.427	28.15		8.0
					21.43	63.172	97.1	0.6	66.044	47.647	35.77		4.7
32.52	66.180	80.9	0.8	39.5	3.70	15.652	19.1	2.0	81.831	113.732	8.92		12.7
					48.25	185.404		0.4	185.404	381.981		33.49	6.94
					5.00	17.662		1.0	17.662	15.923		4.20	1.10
						76.525			76.525	65.074		32.00	5.42
					2.94	8.477		1.6	8.477	22.076		2.47	1.41
					1.64	4.834		0.1	4.834	0.345		1.38	0.25
					2.60	7.664		0.1	7.664	0.736		2.19	0.38
					0.00	0.000		0.0	0.000	0.000		0.00	0.00
					1.80	5.236		0.2	5.236	0.641		1.51	0.31
78.15	130.891	18.9			560.821	81.1			691.712	831.853		77.24	8.997888

Run#: P960314CF Based on average feedstock composition  
Date: 4126196  
Time: 10:00

Run Conditions:	Hydrolyzer Temp (C) :	Flash Tank Temp. (C):	98
	Hydrolyzer Residence Time (min) :		
	Hydrolyzer Add Concentration (%):		

Carbon Balance: SSF

Carbon In																		
Component	In Feedstock						In Inoculum						In Enzyme			Total		
	In Raw Feed						In Feed Liquid											
	(% dry wt)	(C-mole/h)	(% Total In)				(g/L)	(C-mole/h)	(% Total In)	(g/L)	(C-mole/h)	(% Total In)	(g/L)	(C-mole/h)	(% Total In)	(C-mole/h)		
	SD Corrip Sqr Error										SD Corrip Sqr Error						Sqr Error	
Cellulose							0.00	0.000		0.000	0.000	#DIV/0!				0.000	0.000	
Glucose	44.62	372487	99.4	10	1229.2	0.00	0.000	0.0	0.0	0.000	0.0		323.0	2.299	0.6	374.786	1229.233	
Galactose	3.63	30303	100.0	0.5	239	0.00	0.000	0.0	0.0	0.000	0.0					30.303	23.892	
Mannose	0	0.000	#DIV/0!	0.0		0.00	0.000	#DIV/0!	0.0	0.000	#DIV/0!					0.000	0.000	
Xylose	18.39	153.519	100.0	2.2	503.4	0.00	0.000	0.0	0.0	0.000	0.0					153.519	503.355	
Arabinose	12.13	101.261	100.0	1.2	1726	0.00	0.000	0.0	0.0	0.000	0.0					101.261	172.600	
Lignin	7.5	89.846	100.0	0.7	1272	0.00	0.000	0.0	0.0			0.0				89.846	127.195	
Ethanol										154.300	32.354	100.0	28.5	2116		32.354	211.625	
Cell Mass										0.000	0.000	#DIV/0!				0.000		
Carbon Dioxide																		
Glycerol						0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!					0.000		
Acetic Acid						0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!					0.000		
Lactic Acid						0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!					0.000		
Succinic Acid						0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!					0.000		
Xylitol						0.00	0.000	#DIV/0!	0.000	0.000	#DIV/0!					0.000		
Total	86.27	747.416	95.6		2056.2		0.000	0.0								782.069	2056.275	
Standard Dev.	Std. Dev. = 6.21%																	

Inlet Gas Flow Rate		Ethanol		CO <sub>2</sub>		Cellulose Conversion: 85.1% Xylan Conversion: 96.1% Overall C6-Sugar Conversion: 66.9% Overall C5-Sugar Conversion: 31.2% Ethanol Process Yield (% theor): 42.9% Ethanol Metabolic Yield (% theor): 76.2%
Feed moles/min	SD Flow	(mole%)	SD	(mole%)	SD	
0.78	0.078	1.38	0.04	96.78	0.28	
0.16	0.016	1.71	0.04	89.74	0.51	
3.21	0.321	1.3	0.07	2.39	1.9	
0	0	0	0	0	0	

Carbon Out											Conversion	Yield	Std.
In Solids			In Slurry			In Exhaust Gas			Total		(In-Out)/In	g product/ 100 g C6/C5 cons	Deviation
(% dry wt)	(C-mole/h)	(% Total Out)	(g/L)	(C-mole/h)	(% Total Out)	(C-mole/h)	(% Total Out)	(C-mole/h)	(C-mole/h)	(%)			
SD Comp. Sqr. Error					SD Comp. Sqr. Error								
								Sqr. Error	Sqr. Error				
39.25	55.662	52.2	0.8	27.1	2.23	6.920	0.0	0.529	6.920	0.529			
0.80	1.135	5.6	0.1	0.0	17.32	51.056	47.8	0.2	106.718	56.238	71.53		1.50
0.00	0.001	100.0	0.0	0.0	6.51	19.190	94.4	0.2	20.325	4.445	32.93		1.48
4.26	6.041	5.5	0.2	0.4	0.00	0.000	0.0	0.0	0.001	0.000			
1.32	1.872	2.9	0.2	0.1	35.37	104.264	94.5	0.5	110.306	122.612	28.15		7.99
					21.43	63.172	97.1	0.6	65.044	47.309	35.77		4.67
32.52	66.180	80.9	0.8	39.1	3.70	15.652	19.1	2.0	81.831	113.425	8.92		12.62
					48.25	185.404		0.4					
					5.00	17.662		1.0	192.032	382.323		34.94	5.36
									17.662	15.923		4.20	1.10
					2.94	8.477		1.6	58.511	34.879		24.46	4.08
					1.64	4.834		0.1	8.477	22.076		2.47	1.41
					2.60	7.664		0.1	4.834	0.345		1.38	0.25
					0.00	0.000		0.0	7.664	0.736		2.19	0.38
					1.80	5.236		0.2	0.000	0.000		0.00	0.00
									5.236	0.641		1.51	0.31
78.15	130.891	19.2			484.296	71.2			680.326	801.483		71.16	6.99

## Appendix C

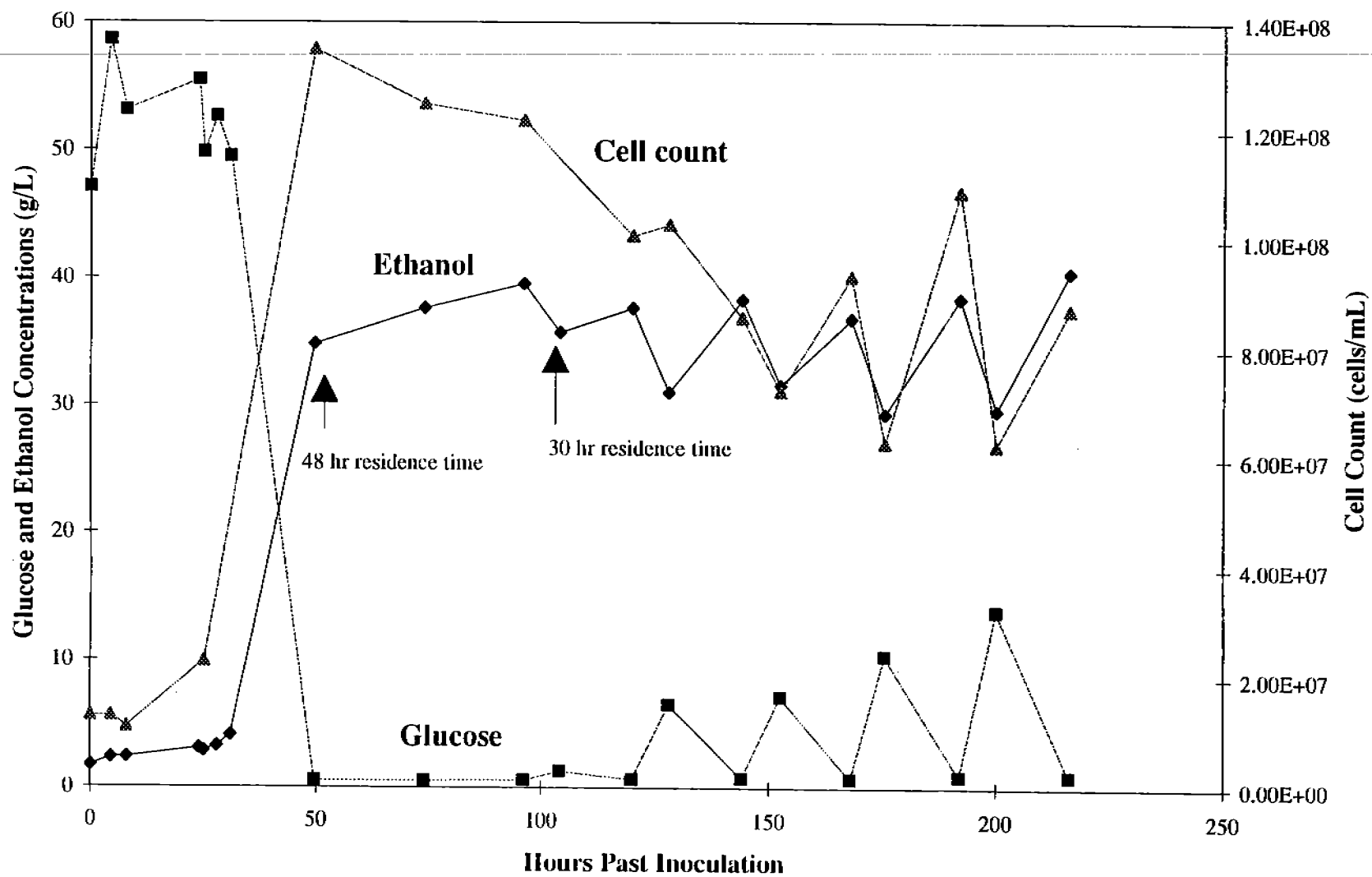
### Continuous Inoculum Study

Providing continuous inoculum to a corn fiber fermentation is an added expense in capital, in raw material costs and in operating labor. If nutrients are supplied at a steady rate and the yeast are not inhibited by toxins or being washed out, then the cell concentration should stabilize. The recombinant yeast should be able to achieve a steady state cell concentration on glucose in absence of any toxins inhibitory to glucose metabolism. This should be possible in the PDU since ethanol concentrations are below inhibitory levels and the dilution rate is well below the maximum growth rate of the organism.

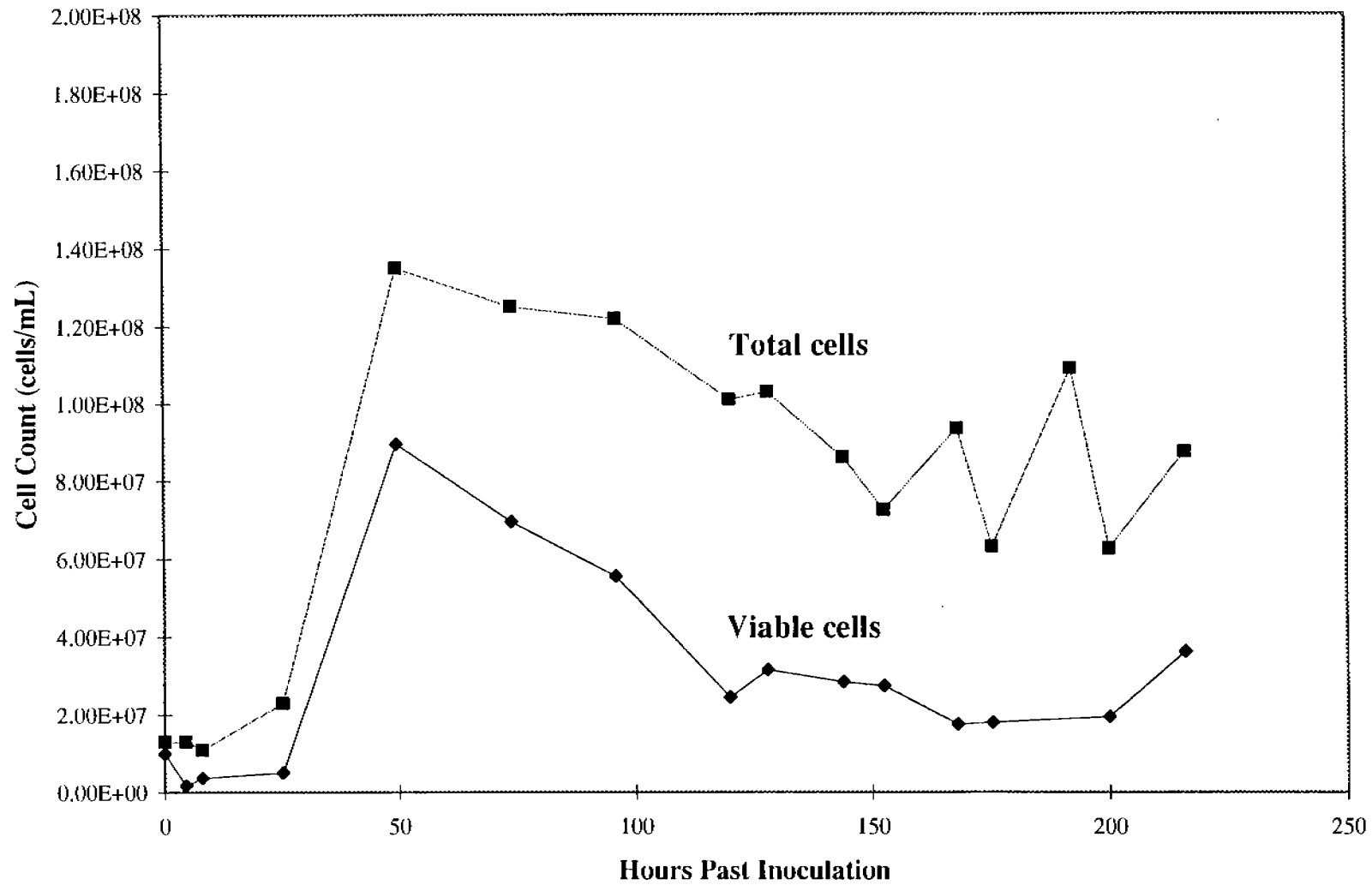
Before eliminating the continuous inoculum in the PDU, a small scale draw and fill fermentation was conducted to demonstrate that a steady state cell concentration could be achieved without continuously feeding cells to the fermenter. A 25% solids, pretreated corn-fiber blend was manually fed to a small scale flask twice daily at a 30 hour residence time. Both cellulase and glucoamylase were added to each fill at 10 IU/ g cellulose and 2 IU/g starch, respectively. CSL at 1.0% ( w/w) was also added at each fill. Initially, a 10% (w/w) yeast inoculum (LNHST2) was added to the fermenter. Fermentation temperature was 30°C and the flask was mixed at 100 rpm in a gyrotory shaker. The pH was controlled at 5.0 with 3 M sodium hydroxide. Figure C-1 shows ethanol and glucose concentration, as determined by YSI, and cell concentration in the fermentation. The lag at the beginning of the fermentation was due to autoclaving corn steep liquor and the pretreated corn fiber together. The same problem occurred during Task 3 batch fermentations. 1.0% CSL was added to the fermenter after 24 hours to ensure the yeast had enough nutrients for growth. At 31 hours into the fermentation, the yeast began to consume glucose and produce ethanol. After additions were adjusted to achieve a 30 hour residence time, the cell counts stabilized and remained steady for three more volume changes (90 hours) without adding cells. Ethanol concentration also reached a steady value. The results from this experiment proved that continuous inoculation could be eliminated in Task 4. This draw and fill scenario may be useful for other bench scale experiments when solids must be used.

One interesting result from the draw and fill fermentation was the discrepancy between the total number of cells counted on a hemacytometer and the number of viable yeast counted on agar plates. Previously, viable cell counts were used to monitor cell population and estimate cell mass. Because the corn fiber produced during task 4 was well pretreated, it was possible to count the actual number of yeast present in the fermenter using a microscope and hemacytometer. Figure C-2 shows the large difference between hemacytometer cell counts and viable cell counts (agar plate). Viable cell counts underestimate cell mass. A correlation will be developed for estimating cell mass from hemacytometer cell counts during Task 5. This will help close mass balances around fermentation.

**Figure C-1. Shake Flask Draw and Fill Experiment**



**Figure C-2. Total and Viable Cell Counts**



## Appendix D

### Effect of Nutrients, Acetic Acid, Sugar Levels, pH, and Neutralizing Agent on Ethanol Yields and Xylose Conversion with LNHST2

#### D.1 Objective

A two-level, three factor, half fractional factorial experiment was designed as a screening study to examine the effect of nutrient types (CSL - 1% w/v versus 2% w/v, and 2% w/v CSL versus YEP), total sugar concentration (82.6 g/L versus 115.5 g/L), and acetic acid concentration (3.3 g/L versus 6.2 g/L), on ethanol metabolic and process yields, and sugar consumption.

A second two-level, four factor, half fractional factorial experiment was designed and carried out to examine the effect of CSL (1% w/v versus 2% w/v), initial total sugar levels (74.4 g/L versus 105.8 g/L), pH (5 versus 6.5) and the caustic (ammonium hydroxide,  $\text{NH}_4\text{OH}$  versus sodium hydroxide,  $\text{NaOH}$ ) on fermentation performance.

#### D.2 Materials and Methods

##### D.2.1 Inoculum Preparation

For each experiment, inoculum was generated in two stages at a controlled temperature of 30°C and 150 rpm mixing. The first stage, consisting of 50 mL of YEPD (1% w/v yeast extract, 2% w/v peptone and 2% w/v glucose, pH 5) in a 250 mL baffled Erlenmeyer flask, was inoculated with 1 mL of LNHST2 from a frozen seed stock. After a 13 hour incubation, a 10% v/v inoculum was transferred to a second stage consisting of CSL medium (1% w/v CSL, 2% w/v glucose, pH 5). The second stage was incubated for only 6 hours by which time a majority of the glucose is consumed, but not all (exponential growth stage). A 10% v/v inoculum from the second stage was used to inoculate the flasks in both experiments.

##### D.2.2 Preparation of Liquor Hydrolyzate

These studies were carried out in shake flasks using hydrolyzate separated from a mixture of pretreated corn fiber and corn screenings (collected on 4/21/96). Liquor was separated from pretreated material by vacuum filtration. The pH of the collected liquor fraction was adjusted to 5.0 with sodium hydroxide pellets. After pH adjustment, the liquor was centrifuged at 9000 rpm for 30 minutes and serially filtered through 0.45 m and 0.2 m filter units to sterilize. The amount of hydrolyzate used in each flask was equivalent to the amount present in a 25% total solids fermentation.

##### D.2.3 Flask Set-up

Exogenous glucose, xylose and acetic acid (filter sterilized solutions) were added to some of the flasks. Solutions of 10% CSL at pH 5 and pH 6.5 were prepared and sterilized. A 10x solution of yeast extract and peptone (YEP), pH 5, was prepared and filter sterilized, for those flasks requiring YEP as the nutrient source (final concentrations in the flasks at 1% w/v yeast extract and 2% w/v peptone). The pH of each flask was monitored and adjusted to pH 5 with 3 M  $\text{NaOH}$  in the first experiment and to pH 5 or 6.5 with either  $\text{NaOH}$  or  $\text{NH}_4\text{OH}$  in the second experiment. The temperature was maintained at 30°C and mixing was controlled at 150 rpm. Table D-1 and D-2 depicts the levels of each factor used in both experiments. No enzymes were used in these experiments.

Table D-1. Design of Experiment 1:  
Two-level, Three Factor, Half-Fractional Factorial Experiment

Flask #	Nutrients	Acetic acid	Total Sugar
CSL			
1	1 (-)	3.3 (-)	115.5 (+)
2	2 (+)	3.3 (-)	82.6 (-)
3	1 (-)	6.2 (+)	82.6 (-)
4	2 (+)	6.2 (+)	115.5 (+)
5	1.5	4.8	96.7
6	1.5	4.8	96.7
YEP			
7	1 (-)	3.3 (-)	115.5 (+)
8	1 (-)	6.2 (+)	82.6 (-)

Table D-2. Design of Experiment 2:  
Two-level, Four Factor, Half Fractional Factorial Experiment

Flask	CSL	Sugar	pH	Base
1	1 (-)	74.4 (-)	5 (-)	NH <sub>4</sub> OH (-)
2	2 (+)	74.4 (-)	5 (-)	NaOH (+)
3	1 (-)	105.8 (+)	5 (-)	NaOH (+)
4	2 (+)	105.8 (+)	5 (-)	NH <sub>4</sub> OH (-)
5	1 (-)	74.4 (-)	6.5 (+)	NaOH (+)
6	2 (+)	74.4 (-)	6.5 (+)	NH <sub>4</sub> OH (-)
7	1 (-)	105.8 (+)	6.5 (+)	NH <sub>4</sub> OH (-)
8	2 (+)	105.8 (+)	6.5 (+)	NaOH (+)

### D.3 Results and Discussion

#### D.3.1 First Shake Flask Experiment

Glucose was consumed in all of the flasks within 18 hours after inoculation. After 66 hours, complete xylose conversion was not observed in any of the flasks. The best conversion of xylose and the best ethanol yields (process and metabolic) were observed in the flasks that did not have additional acetic acid (flasks 1,2 and 7) (see Table D-3 and Figure D-1). Figure D-1 shows the inhibitory effect of acetic acid on both xylose conversion and the ethanol process yield. The conversion of xylose is almost half that in the flasks containing 6.2 g/L acetic acid compared to the flasks containing 3.3 g/L acetic acid. These results correlate with the results of experiments performed at Purdue University (see Bench Scale Experiment 1.9 Report, Acid and Ethanol Inhibition of LNHST2).

Based on the statistical analysis of the results, acetic acid was the only variable that had a major effect in this experiment (see Figure D-2 and D-3). The concentration of CSL and initial sugars did not have an effect on the overall xylose conversion or ethanol yields. There was no improvement in fermentation performance by increasing CSL from 1% w/v to 2% w/v (see Figure D-2). Adding YEP as the nutrient source also did not increase the conversion of xylose or the ethanol yields (see Table D-3 and Figure D-1).

Table D-3. Results of First Shake Flask Experiment

Flask		Glucose Conversion (%)	Xylose Conversion (%)	Ethanol Process Yield (% theoretical)	Ethanol Metabolic Yield (% theoretical)
1	CSL	100	75.57	74.79	83.20
2		100	73.27	79.78	90.05
3		100	46.17	58.16	75.54
4		100	38.26	57.26	76.86
5	Centerpoints	100	53.93	65.54	81.22
6		100	53.24	64.96	80.75
7	YEP	100	62.21	68.07	80.66
8		100	43.61	57.30	75.50

### D.3.2 Second Shake Flask Experiment

The purpose of the second experiment was to examine the effect of caustic type on ethanol process yield and xylose conversion. Xylose conversion was improved with both NaOH and  $\text{NH}_4\text{OH}$  at a pH of 6.5. However, the increased xylose conversion did not translate into an increased ethanol yield when  $\text{NH}_4\text{OH}$  was used for neutralization and pH control due to production of higher levels of the by-products xylitol (may include arabitol), glycerol, acetic acid and cell mass (see Table D-4 and Figure D-4). The process yield was better in all cases when NaOH was used for pH control, and was slightly better at pH 6.5 than pH 5 (see Figure D-5). Again, at the higher pH, more by-products were produced than at the lower pH. As in the first experiment, a concentration of 2% w/v CSL did not help fermentation performance.

The statistical analysis performed on the data showed that 2% w/v CSL did not help improve xylose conversion over 1% w/v CSL (same conclusion drawn in first experiment) (see Figure D-6). The initial sugar level also did not have an affect on the conversion of xylose, but a higher initial sugar level did slightly decrease the ethanol yield.

An interesting phenomena observed in this experiment was the utilization of arabinose at the higher pH level (see Table D-4). This could be due to the presence of a contaminant, but contamination was not observed in any of the flasks. With the conversion of arabinose, a large amount of xylitol (probably includes arabitol as it has nearly the same retention time as xylitol) was observed. In addition to xylitol, acetic acid was produced at significant quantities when  $\text{NH}_4\text{OH}$  was used for pH control. If the yeast can in fact use arabinose, we could potentially minimize the contamination problems we have observed in the PDU, which has been observed to utilize arabinose in the past, by operating under conditions that favor arabinose utilization by LNHST2.

Table D-4. Results of Second Shake Flask Experiment

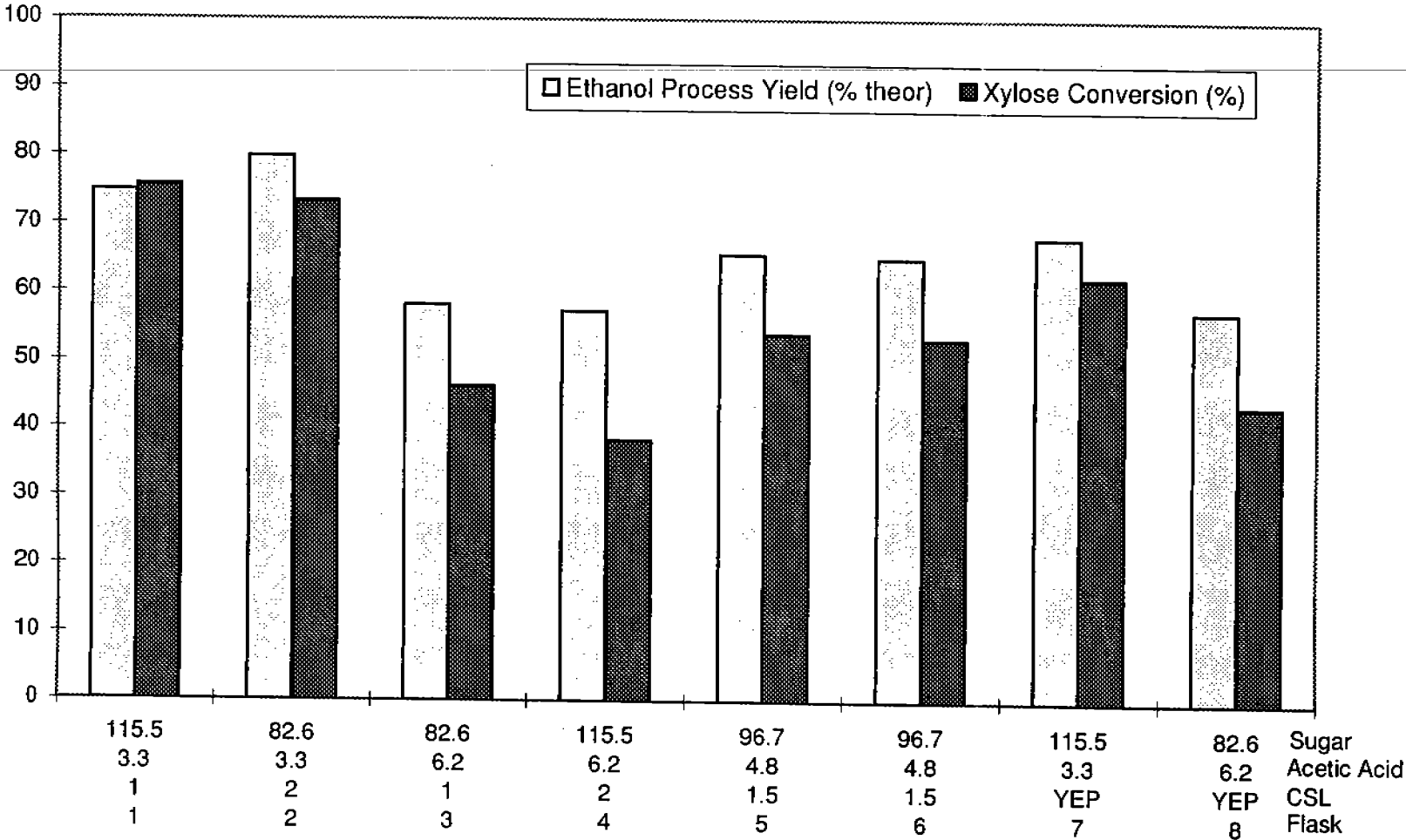
Flask	Glu. Conv.	Xylose Conv.	Ethanol Process Yield	Ethanol Meta. Yield	Arab. Conv.	DCW	Glyc. Prod.	Xylit. Prod.	Acetic Acid
	(%)	(%)	(%)	(%)	(%)	(g/L)	(g/L)	(g/L)	(g/L)
1	100	69.0	79.6	91.4	13.3	6.0	1.8	2.3	0.7
2	100	83.4	85.5	92.4	14.7	7.2	2.2	2.8	0.0
3	100	84.2	83.2	89.3	12.9	7.6	3.4	2.3	0.0
4	100	61.9	71.0	83.8	7.4	6.7	3.4	1.9	1.0
5	100	95.0	92.7	95.9	64.6	12.2	4.0	16.9	1.2
6	100	90.2	78.4	82.8	60.4	8.2	3.5	25.4	7.4
7	100	85.8	66.9	71.5	54.0	8.2	6.1	13.3	8.7
8	100	95.7	86.2	88.6	54.4	11.4	5.8	3.6	1.2

#### D.4 Conclusions

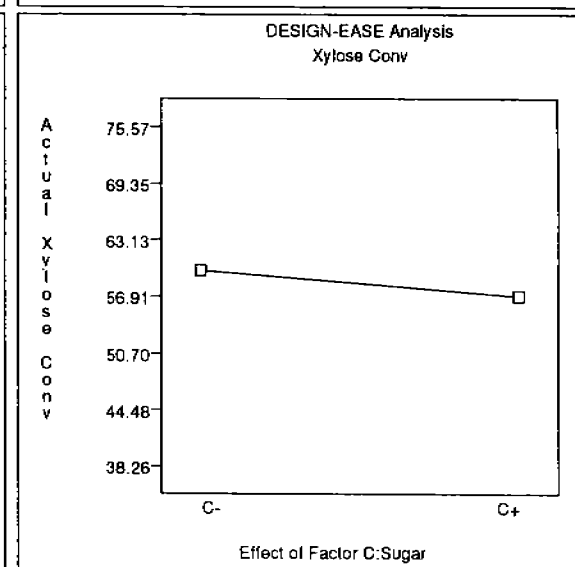
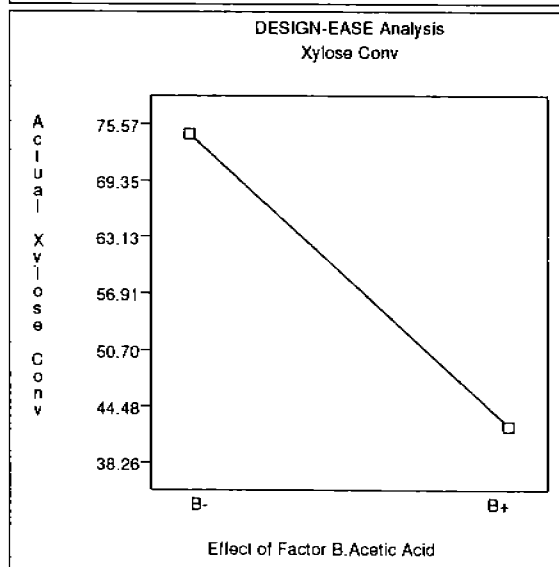
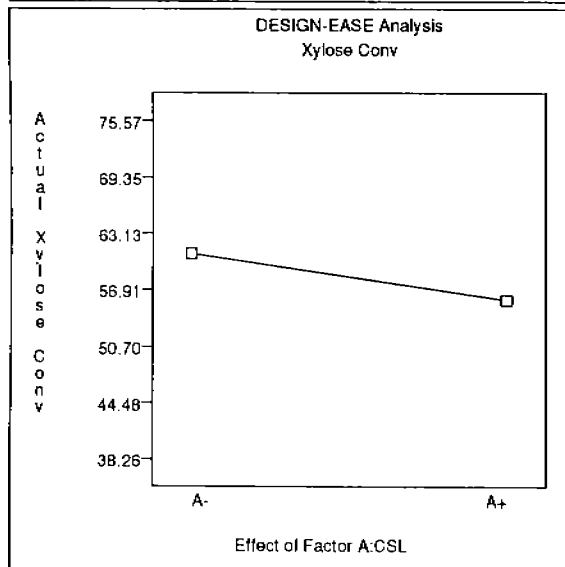
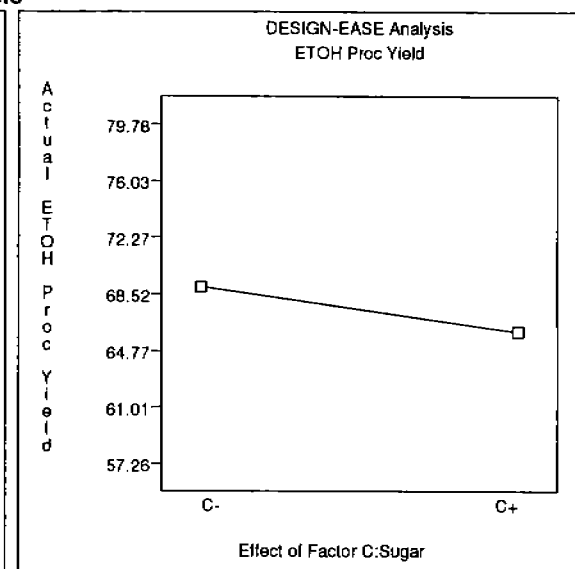
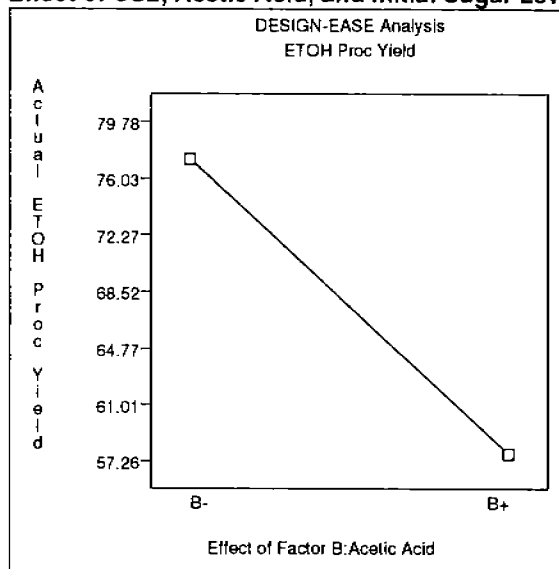
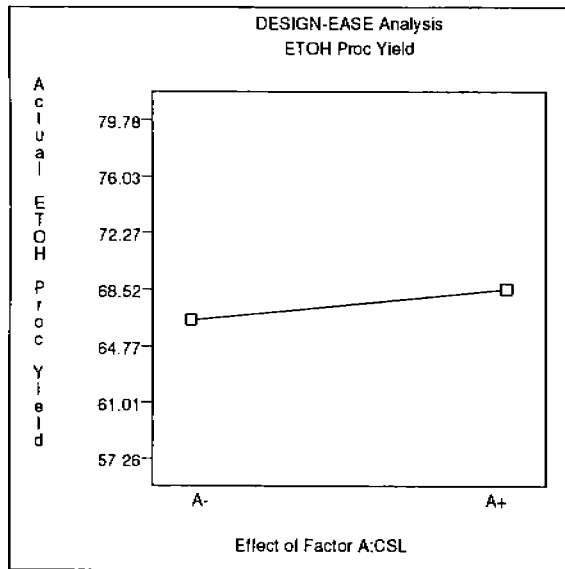
The first shake flask experiment demonstrated the inhibitory affect of acetic acid on the LNHST2 fermentation. Increasing the CSL concentration to 2% w/v did not produce an increase in xylose conversion. In addition, YEP also did not improve xylose conversion over 1% CSL.

The second experiment showed the strong negative effect of using  $\text{NH}_4\text{OH}$  for pH adjustment. At a pH of 5, poor xylose conversion was observed with  $\text{NH}_4\text{OH}$ . At a pH of 6.5, xylose conversion improved, but the ethanol yield did not improve because of greater by-product production. At pH 5 with NaOH, xylose conversions and ethanol yields were just over 80%. At pH 6 with NaOH, the xylose conversions and ethanol yields were slightly better than pH 5. But operating at a higher pH may not justify a higher risk of contamination. Increasing the concentration of CSL and the initial sugar levels did not have a significant effect on xylose conversion and ethanol yield.

Figure D-1. Ethanol Process Yields and Xylose Conversions with LNHST2  
(Experiment 1)



**Figure D-2. Statistical Analysis of Experiment 1:  
Effect of CSL, Acetic Acid, and Initial Sugar Levels**



**Figure D-3 Statistical Analysis of Experiment 1:  
Effect of Nutrient Type, Acetic Acid, and Initial Sugar Levels**

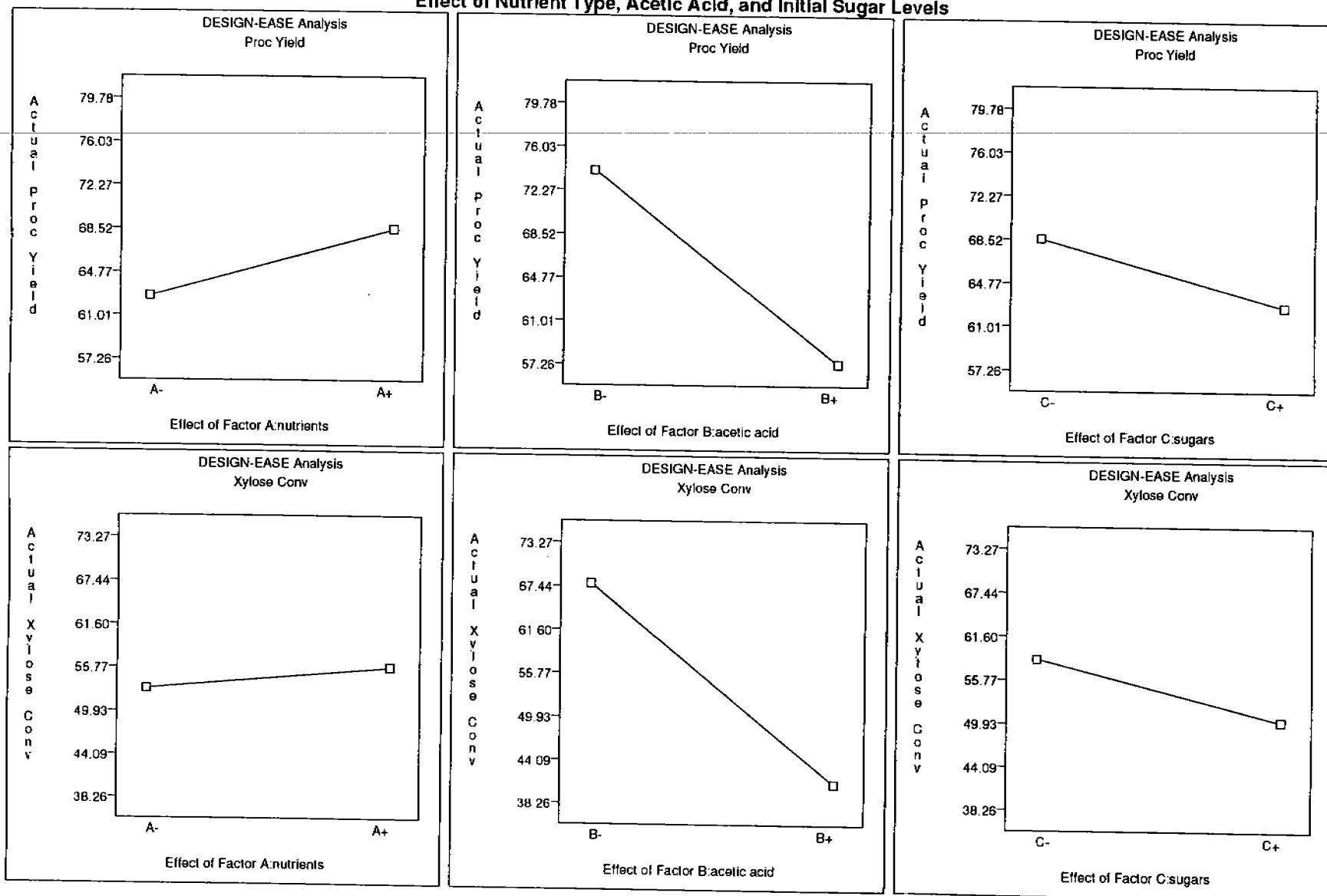
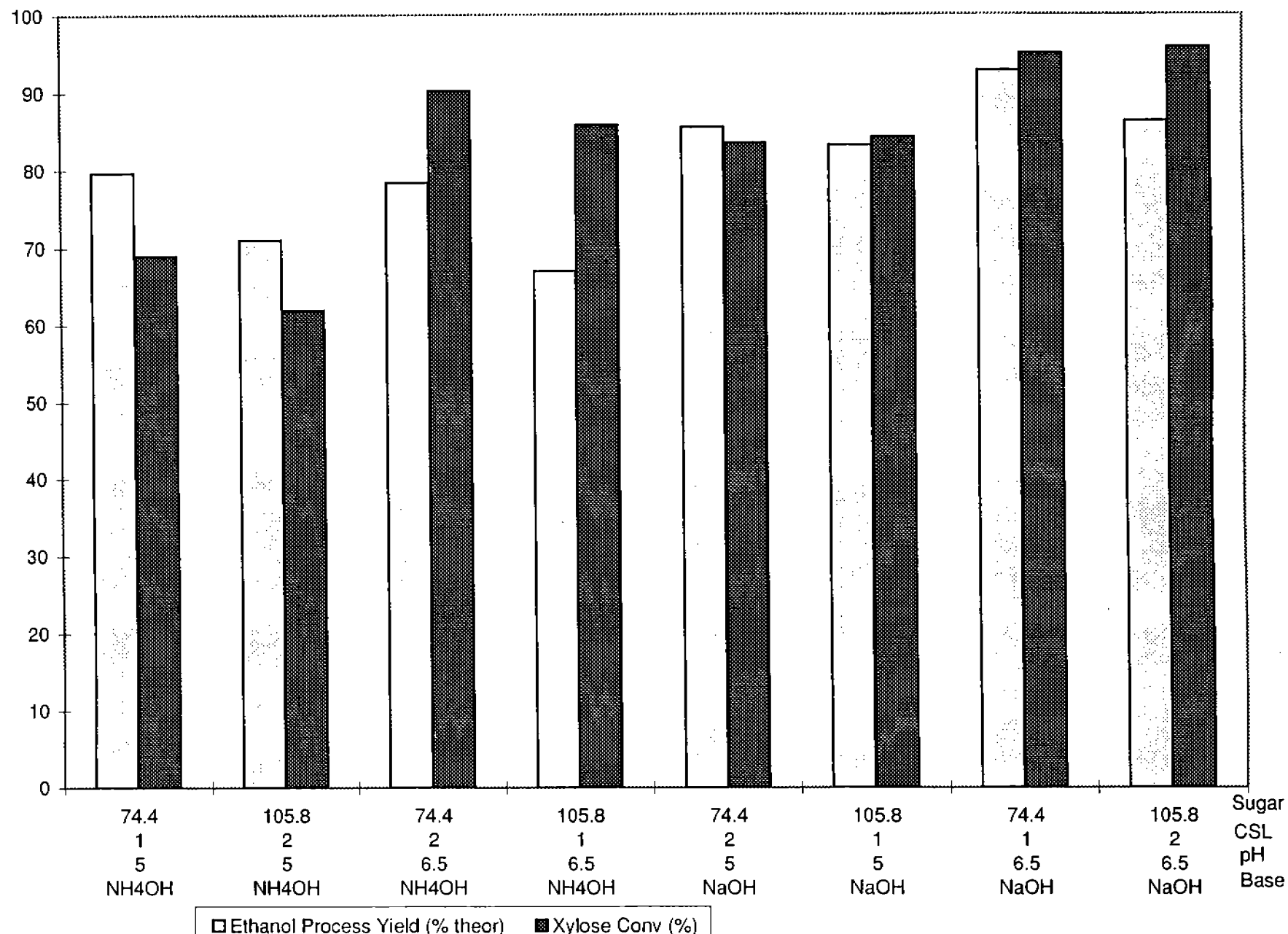
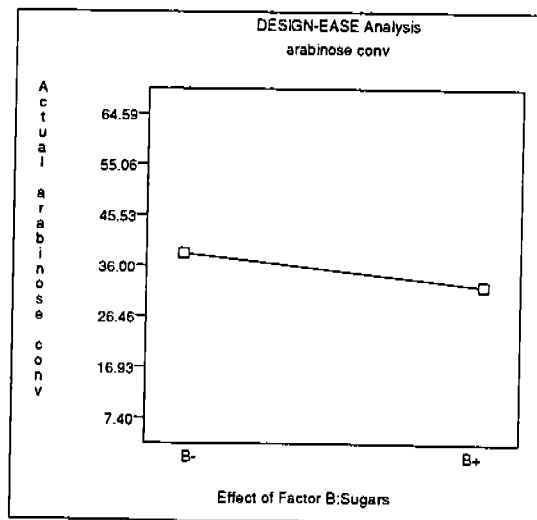
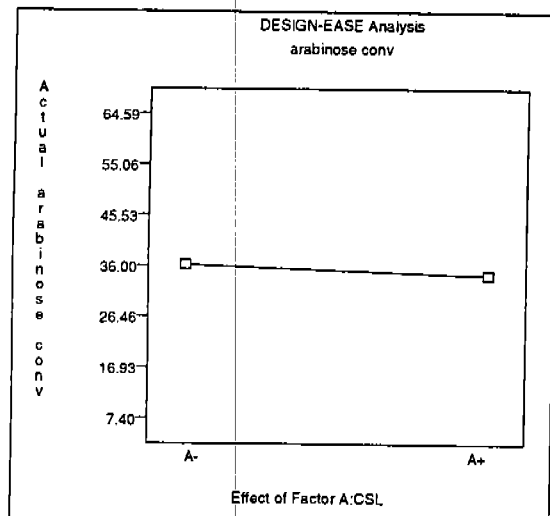
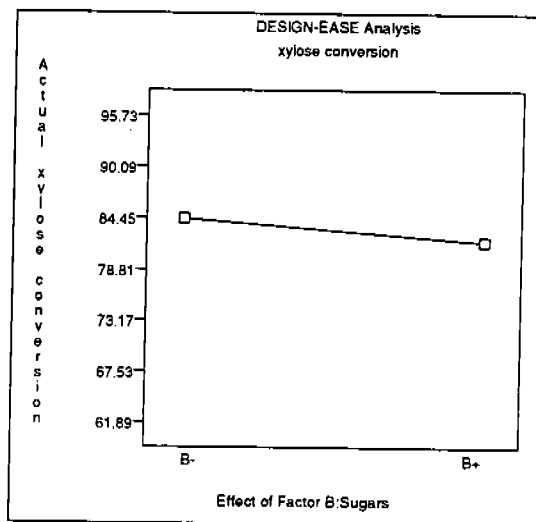
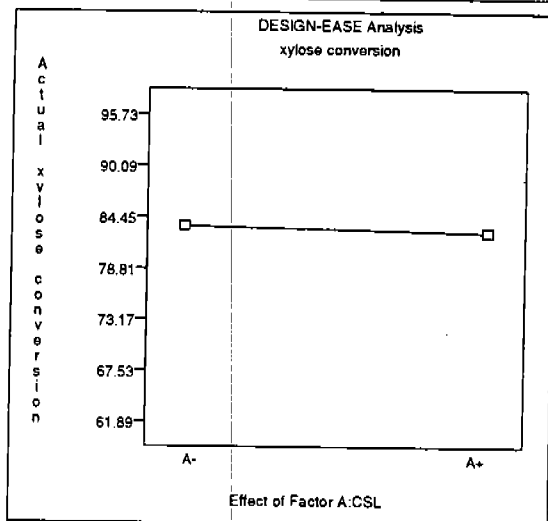
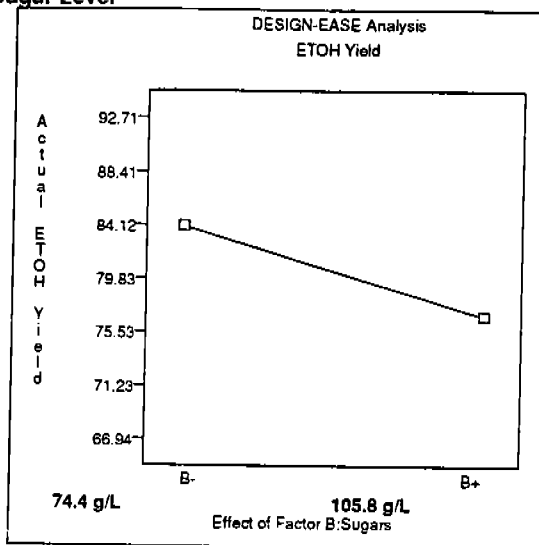
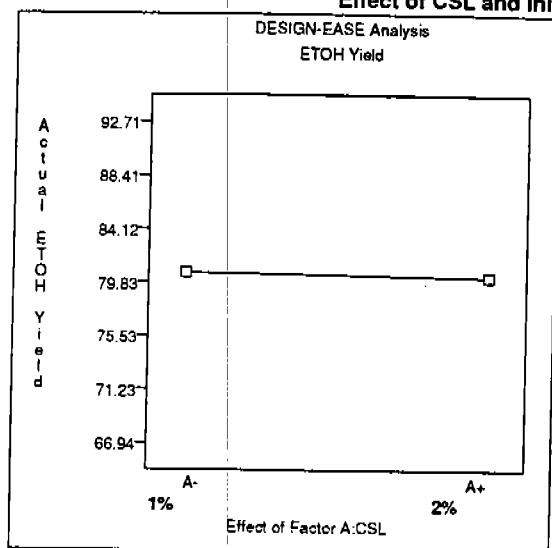


Figure D-4. Ethanol Process Yields and Xylose Conversions with LNHST2 (Experiment 2)



**Figure D-6. Statistical Analysis of Experiment 2:  
Effect of CSL and Initial Sugar Level**



# Experiment 1: Raw Data

Flask	1									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0.00	67.81	47.83	25.11	0.00	2.07	0.00	3.28	0.81	0.47	
3.00	65.47	47.37	24.88	0.00	2.03	0.00	3.26	1.43		
6.00	60.38	47.15	24.86	0.00	2.22	0.52	3.26	3.41		
18.00	0.00	38.59	25.90	0.00	2.25	3.27	2.57	34.07		
25.50	0.00	33.67	26.04	0.00	2.24	3.42	2.57	35.36		
42.00	0.00	21.50	25.70	0.00	2.32	3.76	2.68	41.49	8.03	
66.00	0.00	11.68	25.38	0.00	2.06	3.70	2.63	45.01	8.77	
Flask	2									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	47.04	35.06	24.92	0.00	3.02	0.00	3.31	0.91	1.40	
3	45.11	35.04	25.00	0.00	3.29	0.32	3.32	1.64		
6	38.29	34.13	24.60	0.00	3.27	0.61	3.23	4.04		
18	0.00	27.35	25.63	0.00	3.29	2.45	2.85	24.71		
25.5	0.00	24.76	26.73	0.00	3.40	2.63	3.04	28.37		
42	0.00	15.37	25.40	0.00	3.20	2.55	3.05	32.89	7.10	
66	0.00	9.37	25.34	0.00	3.04	2.64	3.47	34.37	7.47	
Flask	3									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	47.56	35.49	25.23	0.00	2.11	0.00	6.17	0.84	0.53	
3	45.22	34.86	24.84	0.00	2.04	0.00	6.07	1.48		
6	40.72	34.34	24.66	0.00	2.20	0.39	6.02	3.05		
18	0.00	29.31	25.60	0.00	2.23	2.14	5.61	23.53		
25.5	0.00	27.21	25.44	0.00	2.22	2.17	5.69	23.72		
42	0.00	24.24	24.74	0.00	2.22	2.24	6.09	24.90	5.07	
66	0.00	19.11	25.36	0.00	2.14	2.29	6.48	25.53	5.73	
Flask	4									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	67.76	47.69	25.10	0.00	3.04	0.00	6.14	0.87	1.40	
3	66.33	47.35	24.91	0.00	2.98	0.00	6.12	1.33		
6	63.30	47.35	24.98	0.00	2.93	0.00	6.13	2.35		
18	0.19	41.33	25.68	0.00	3.27	3.04	5.61	32.73		
25.5	0.00	38.33	25.60	0.00	3.24	3.17	5.60	32.78		
42	0.00	34.46	25.87	0.00	3.24	3.26	6.03	34.02	5.90	
66	0.00	29.44	25.77	0.00	3.15	3.31	6.62	34.65	6.43	
Flask	5									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	55.83	40.26	24.25	0.00	2.50	0.00	4.76	0.77	0.93	
3	55.50	41.19	24.85	0.00	2.54	0.00	4.89	1.46		
6	50.31	40.58	24.64	0.00	2.73	0.49	4.82	3.30		
18	0.00	34.13	25.50	0.00	2.75	2.67	4.24	28.22		
42	0.00	25.79	25.61	0.00	2.71	2.80	4.53	31.62	6.00	
66	0.00	18.55	25.23	0.00	2.68	2.87	4.89	32.95	6.67	
Flask	6									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	56.66	40.72	24.57	0.00	2.45	0.00	4.84	0.76	0.87	
3	56.28	41.78	25.20	0.00	2.47	0.00	4.98	1.45		
6	50.33	40.32	24.53	0.00	2.67	0.47	4.80	3.15		
18	0.00	34.74	25.88	0.00	2.72	2.65	4.33	28.71		
42	0.00	26.33	25.66	0.00	2.63	2.73	4.44	31.57	6.17	
66	0.00	19.04	25.30	0.00	2.55	2.80	4.75	33.09	6.87	
Flask	7									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	67.26	47.31	24.82	0.00	1.15	0.00	3.23	0.74	0.87	
3	65.99	47.66	24.99	0.00	1.24	0.30	3.29	1.36		
6	61.31	47.33	34.95	0.00	1.25	0.55	3.27	3.04		
18	0.00	39.17	26.05	0.00	1.24	3.50	2.79	34.12		
42	0.00	26.15	25.36	0.00	1.17	3.61	2.87	38.49	7.67	
66	0.00	17.88	25.14	0.00	1.13	3.62	3.62	40.59	8.83	
Flask	8									
	Glucose	Xylose	Arabinose	Succinic	Lactic	Glycerol	Acetic	Ethanol	DCW	
Time (h)	(g/L)	(g/L)	(g/L)	acid (g/L)	acid (g/L)	(g/L)	acid (g/L)	(g/L)	(g/L)	(g/L)
0	47.01	35.13	24.98	0.00	1.17	0.00	6.19	0.81	0.73	
3	45.02	34.65	24.67	0.00	1.21	0.24	6.12	1.47		
6	41.16	34.34	24.63	0.00	1.22	0.43	6.09	2.78		
18	0.00	29.41	24.50	0.00	1.23	2.38	5.77	23.26		
42	0.00	24.55	25.31	0.00	1.19	2.48	6.29	24.08	7.67	
66	0.00	19.81	25.18	0.00	1.10	2.46	6.70	24.86	6.03	

# Raw Data: Experiment 2

Flask	1							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	47.11	27.54	22.66	1.08	2.47	0.73	3.98	0.78
19	1.37	22.09	22.00	1.12	2.53	2.60	3.48	23.62
48	1.03	14.30	20.79	1.51	2.46	2.66	3.68	28.80
68	1.14	11.14	19.95	1.56	2.31	2.34	3.84	30.89
91	1.04	8.55	19.65	3.37	2.42	2.52	4.69	31.16
Flask	2							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	46.82	27.40	22.38	0.95	3.17	0.51	3.83	0.77
19	1.41	21.84	21.73	0.99	3.24	2.49	3.37	23.72
48	1.05	12.20	20.71	1.33	2.91	2.29	3.16	29.69
68	1.17	7.63	19.62	1.65	3.07	2.52	3.45	32.88
91	1.04	4.54	19.09	3.79	2.94	2.70	3.52	33.19
Flask	3							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	66.74	39.39	22.80	0.88	2.20	0.36	3.75	0.80
19	1.30	33.04	22.65	0.98	2.36	3.36	3.63	33.56
48	1.06	3.49	20.96	1.39	2.18	3.32	2.72	40.03
68	1.16	11.16	19.86	1.75	2.26	3.71	2.88	44.94
91	1.09	6.24	19.23	3.23	2.12	3.63	2.76	45.90
Flask	4							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	66.31	39.16	22.69	0.94	3.14	0.47	3.77	0.81
19	1.32	33.40	22.73	1.03	3.30	3.52	3.09	33.97
48	1.05	22.15	21.64	1.21	3.04	3.36	3.17	37.87
68	1.19	17.88	20.62	1.41	3.08	3.69	3.82	39.40
91	1.23	14.93	21.01		2.91	3.83	4.74	39.07
Flask	5							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	45.71	26.37	21.05	0.87	2.17	0.37	3.77	0.81
19	1.17	17.67	21.00	1.16	2.17	3.92	3.75	26.42
48	0.32	3.90	11.24	11.91	1.80	4.30	4.48	34.48
68	1.21	1.86	8.84	16.07	1.70	4.61	5.09	35.60
91	1.07	1.33	7.46	17.81	1.49	4.39	5.01	34.96
Flask	6							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	44.47	26.15	21.31	0.93	3.08	0.46	3.80	0.81
19	1.30	17.95	21.54	1.10	3.12	3.63	3.96	26.53
48	0.76	7.02	12.66	10.25	2.73	4.00	6.97	31.22
68	1.23	4.41	10.46	13.99	2.69	4.33	9.30	30.87
91	1.21	2.56	8.45	16.32	2.34	3.99	11.18	29.08
Flask	7							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	67.25	39.98	23.18	0.90	2.18	0.37	3.75	0.79
19	1.25	30.41	22.37	0.99	2.30	5.38	4.00	32.82
48	1.02	11.99	15.46	7.83	2.05	6.25	7.06	39.14
68	1.21	8.38	12.54	11.35	1.93	6.26	9.24	38.10
91	1.18	5.67	10.67	14.15	1.86	6.44	12.41	37.47
Flask	8							
	Glucose	Xylose	Arabinose	Xylitol	Lactic acid	Glycerol	Acetic acid	Ethanol
Time (h)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)	(g/L)
0	67.26	39.82	22.95	0.93	3.05	0.46	3.77	0.78
19	1.23	20.90	21.85	1.08	3.28	5.23	3.63	33.05
48	1.04	7.56	15.82	7.70	3.04	5.98	4.14	44.77
68	1.29	3.27	12.43	11.53	2.95	6.14	4.95	46.81
91	1.18	1.70	10.47	14.51	2.88	6.26	5.59	47.95